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RELATIONSHIP BETWEEN OTOLITHS AND NYSTAGMUS

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In rabbits the influence of the function f the otoliths upon vestibular nystagmus was investigated. In a se ries of xperiments the function of otolithic organs was eliminated and thereafter the semicircular canal function was re-examined by various tests. Stimulation of the utricular nerve by either electrical or mechanical stimuli did not produce nystagmus, but movements f the utricular membrane provoked an intense nystagmus, presumably by endolymphe move ments causing cupular deviations.
The literature on the subject is reviewed.

The physiologic stimulus to the otoliths and semicircular canals is well established. The re sponse of each separate sub-organ, on the other hand, is questionable. It is well known that canals respond in nystagmus and otoliths in a compensatory eye movement, if stimulated by angular or linear acceleration respectively The fact that nystagmus can also be provoked by a linear acceleration (e.g. gravity) makes it acceptable that the semicircular canals are not the only vestibular generators of nystagmus, and suggests that also the otoliths can influence the appearance of nystagmus. The basic principles of nystagmus provoked by a linear acceleration are explained as follows by Jongkees & Philipszoon (1962)· Nystagmus consists of two components viz a slow and a fast phase· each of these has its own threshold. When the eye is deviated either by a cupula or an otolith stimulation, the threshold for a quick phase is triggered and a nystagmus beat appears (Alexander's law 1911) the quick phase is then in opposite direction to the slow one The saccadic repositioning endeavours to correct the eye position from an extreme stand to a neutral position.

This work was supported by a C.S I R. grant, South Africa.

METHOD AND PROCEDURES

In order to examine the relationship between otoliths and nystagmus provoked by a linear acceleration, we eliminated selective parts of the labyrinth.

Surgical interventions such as cutting the utricular nerve and elimination of the saccule make it possible to prove that the semicircular canals do not respond to a linear acceleration. It is, however very difficult to perform selec tive operations on the otoliths in order to test or eliminate their function without damaging other parts of the vestibular organ, i.e the canals. It is impossible to state with confidence that a functional alteration of the labyrinth can be confirmed histologically Micro-anatomical integrity does not prove functional normality (James, 1966) For this reason we did not rely on histological techniques. We intended to examine the effects of the otoliths by way of physiological recordings. In how far the otoliths are responsible for nystagmus, can be found out by a step-by-step elimination of various suborgans: first the saccule and then the utricle. Every step of the operation was checked with rotation tests about a vertical as well as a hori-

zontal axis, hence a very light anaesthesia is essential. In our approach toward a selective labyrinthectomy (that is sectioning of the utricular nerve) we encounter a phase where stimulation of the nerve is very easy Although it is known that in special circumstances linear accelerations can give nystagmus (Jongkees & Philipszoon, 1962) it is not clear whether direct electrical or mechanical stimulation (which provokes movements of the otoliths and thus may be regarded as a more or less physiologic stimulus) of the utricular macula or its nerve leads to nystagmus or whether electrical stimulation influences an already present nystagmus. Versteegh (1927) Jongkees (1950) Sullivan *et al.* (1957) denied any development of nystagmus by direct mechanical stimulation of the otolith system

Neither did Ulrich (1935) succeed in provoking nystagmus by direct stimulation of the otoliths with a human hair but Fernández *et al* (1959) found nystagmus when they stimulated (by cutting the nerve) the utricular nerve of cats. From the current data we cannot come to a definite conclusion as to what the effects of artifical stimulation on the utriculus or its nerve are

MATERIALS AND PROCEDURE

The group of de utricularized animals consists of more than fifty rabbits of which only seven were classified as successful on both sides The labyrinth function of each animal was evaluated pre-operatively by a torsion-swing, a parallel-swing, and a barbecue test.[1]

The approach to the inner ear was the same as described by Versteegh (1927) In order to facilitate the sectioning of the utricular nerve, we tried a few techniques with different degrees of success

Touching the utricular nerve with a booklet presumably gives an intense stimulation which causes the animal to start struggling. This might result in a tear of the limiting membrane or damage of the nerve of the lateral semicircular canal, and would eventually lead to failure of the operation. The failure is indicated by a spontaneous nystagmus beating to the normal side, just as in the case of a unilateral total labyrinthectomy

For this reason we proceeded in some cases either by cocainization of the utricular nerve with a few drops of a 5% solution or by paralyzing the animal with flaxedil 1–2 mg/kg of body weight. After removing blood clots and perilymph leaking from the cochlea, the utricular nerve was hooked and cut through under the microscope The booklet was made in our workshop and was not bigger than 100 micron. Thereafter the wound was flushed with a penicillin solution and closed Penicillin was administered intramuscularly for five days post operatively Utricular stimulation was practised on seven rabbits, five times this was successful. In two the utricular macula was damaged during the stimulation because of movements of the animal. The operational approach was the same as for a section of the nerve. A steel electrode was held in position by a micromanipulator (made by Narishige, Japan) so that either the utricle or its nerve could be stimulated separately A Grass stimulator was used to deliver the electrical impulses, and was set at 0.5–0.8 V open-circuit voltage. The pulses lasted 0 1 second and were given at a ratio of five per second. After stimulation of both the macula and the nerve the electrode was moved mechanically up and down on the nerve and the macula over a distance of 100 microns, with the micromanipulator This allows separation of the effects of electrical and mechanical stimulation.

RESULTS

Spontaneous nystagmus resulting from utricular nerve sectioning alone was not seen in the successful cases. This coincides with the results

[1] The barbecue rotation is about a cephalo-caudal horizontal body axis. It is a form of a linear acceleration and provokes a nystagmus superimposed on a compensatory eye movement of otolithic origin (Janeke, 1968).

of Versteegh (1927) Jongkees (1950) Sullivan *et al.* (1957) and Sasaki (1963) If the limiting membrane or the nerve from the lateral semicircular canal was ever so slightly damaged though, spontaneous nystagmus appeared. The nystagmus then beats to the normal side as in the case of a unilateral labyrinthectomy Fernández *et al* (1957) reported spontaneous nystagmus after sectioning the utricular nerve This must be attributed, we think, to damage of the canal system just as in our cases which we classify as non-successful Following the sectioning of the utricular nerve we noticed a tremor of the eyelid, most pronounced in the upper one on the operated side In some cases the membrane nictitans participated. The eye ball, however stood still. The eyelid tremor is clearly recorded by the nystagmograph and can easily be mistaken for eyeball tremors (Hamersma, 1957)

Shirabe *et al* (1965) stimulated the otoliths by centrifugal force and in this way provoked a vertical eyeball tremor (no nystagmus) They ascribed it to eyemuscle cramps" which might be an otolith reflex according to the authors. It might also have been only an eyelid tremor and not an eyeball tremor This confirms the necessity for direct observations. The disturbance of equilibrium after bilateral otolith destruction was characterized by a swaying of the head from left to right and vice versa in a horizontal plane, abduction of the hind limbs and a broad gait. These disorders appeared in all the animals with a selective bilateral loss of utricular function, as well as in animals with a bilateral total labyrinth destruction. It seems that this unstable condition is brought about by the loss of otolith function (Jongkees, 1950)

Before we proceeded to the barbecue rotation we tested again the reactions to both rotatory and linear stimulation. The post-operative cupular responses provoked by the torsion-swing were not identical to the pre-operative recordings (Fig. 1) The reason for this might be microscopial tears in the limiting membrane or a bleeding in the endolymphatic space, such

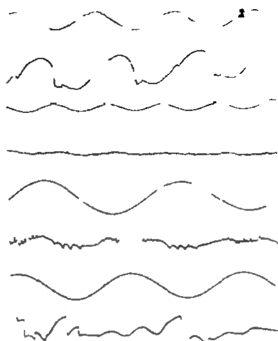


Fig 1 Labyrinth responses following selective labyrinthectomy (on both sides). *1* time in sec *2* Barbecue stimulation *3* torsion-swing stimulation. *A* pre-operativ responses to the barbecue rotation *B* post-operative responses to the barbecue rotation *C* pre-operative responses to the torsion swing; *D* post-operativ responses to the torsion swing.

as were observed in some cases of post-mortem examinations by Miehlke (1955 tension on soft tissues) This change of torsion-swing responses might, at first sight, be attributable to disappearance of otolith influence. But this is not likely since we encountered animals in which torsion-swing recordings were nearly normal. On the parallel-swing no trace of reaction was ever noticed.

During barbecue rotation the only responses were those provoked by the rotatory acceleration resulting in a transitory nystagmus. This is also regarded as a proof of the functionality of the vertical canals (Fig. 2).

During constant rotation we noticed no response at all. The post-rotatory nystagmus provoked in the operated animals could not be qualitatively evaluated—a reason being that

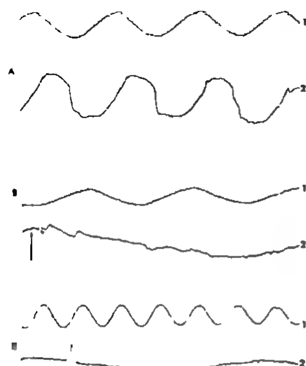


Fig 2 Labyrinth responses following a selective labyrinthectomy on both sides. *A1* Barbecue stimulus (*B1*) *A2* pre-operative responses; *B2* post-operative responses to the barbecue rotation *C1* paralleling stimulus *C2* negative responses to linear accelerations. The squares are calibration waves which indicates that 500 μV = 1 cm on the vertical axis. The arrow indicates the start of the barbecue rotation. A few nystagmus beats provoked by the acceleration re seen in *B2*

the canal responses are not so clearly demarkated as pre-operatively as already mentioned.

If the post-operative post rotatory nystagmus is correlated to the pre-operative responses we could not detect the distinct difference between the prone and supine stop position—the former stop position virtually gave no post-rotatory nustagmus and the latter a strong post-rotatory response. The above finding corroborates the fact that the difference between the 2 positions can be viewed in the light of an otolith modulation effect on cupular responses depending on the orientation of the animal in space.

Concerning utricular stimulation

After the anaesthesia had worn off sufficiently we started our recordings. The salient features of the electrical stimulation were as follows.

No nystagmus was observed by direct observation. In two animals an eye deviation was noticed on the nystagmogram while the rest of the examined animals exhibited a rotatory (frontal) movement of the eyeballs which was not recorded on the nystagmogram. Mechanical pressure on the utricular nerve did not provoke any response. When we moved the microelectrode up and down the utricular macula we observed an intense nystagmus in both eyes. Such a movement simulates in exaggerated form a linear acceleration. After these experiments we destroyed the utricule and the canals, and the typical nystagmus followed, beating with the fast phase to the normal side. We now stimulated the utricular nerve stump. In three animals a suppression of the nystagmus followed. The suppression lasted till a few seconds after the stimulus ceased.

DISCUSSION

ad. Selective Labyrinthectomy

Benjamins & Huizinga (1927 and 1928) removed the superior part of the labyrinth (utricle and canals) on both sides in pigeons and noticed that the counter-rolling of the eyes remained intact. If they removed the inferior part (saccule, cochlea and lagena) counter *rolling was no longer observed.*

Meyer zum Gottesberge & Plester (1965) who touched the saccule with a fine instrument during stapedectomies noticed that the patient experiences a sense of a change of position in relations to "g". By using the same principle of elimination as Benjamins & Huizinga (1927) Philipszoon & v.d. Laarse (1967) also came to the conclusion that the compensatory eye movement originates in the otoliths. They eliminated the saccule and cut the utricular nerve on one side. The contralateral side was

destroyed. We are convinced that this technique provides sufficient accuracy to conclude that both the compensatory eye movements and the concomitant nystagmus during barbecue rotation are provoked by the otoliths.

Here we see that the canals do not respond to linear accelerations and that otolith stimulation can provoke nystagmus. Although on incorrect grounds, Borries in 1922 was the first to mention the otolith-ocular reflex arch. Many years later Jongkees & Philipszoon (1962) proved that a linear acceleration, which is the appropriate stimulus for the otoliths, can provoke nystagmus. From the results of the utricular stimulation we conclude that two factors are of value, namely (1) that nystagmus beats cannot be generated by direct electrical or mechanical stimulation of the utricular nerve, and (2) that with a minimal pressure on the utricular membrane an intense nystagmus results.

We may regard the latter as an otolith reaction but it may also be due to a mechanical displacement of endolymph with a resultant cupula deflexion.

Now that a more positive proof of an otolith-ocular reflex arch exists, we get a better insight into the origin of positional nystagmus. We do not suggest that the physiology of a rabbit is the same as that of humans, neither do we intend to exclude other forms of positional nystagmus, but we only want to point to the importance of the otoliths as one of the possible causes of positional nystagmus.

CONCLUSION

Following a partial labyrinthectomy on both sides in which the utricular nerves were severed, the saccules destroyed, and the canals left intact, the slow eye movement and the concomitant nystagmus that normally accompany barbecue rotation disappeared. This proves that both the slow eye movements and the concomitant nystagmus provoked by barbecue rotation originate from the otoliths (the integrity of the canals was confirmed by a torsion-swing test). It also proves that the canal system is not gravity sensitive, and cannot be regarded as a linear accelerometer at all.

Mechanical stimulation of the utricular macula might corroborate the finding that the otoliths can generate nystagmus.

ZUSAMMENFASSUNG

Bei Kaninchen wurde der Einfluss der Otolithenfunktion auf den Augennystagmus gemessen. In einer Reihe von Experimenten wurden die Otolithenorgane ausgeschaltet und danach die Bogengangswirkung mit Hilfe von verschiedenen Prüfungen untersucht. Elektrische und mechanische Reizung des durchschnittenen Nervus utricularis verursachte keinen Nystagmus, aber Bewegungen der Membrana Utriculi führten zu einem starken Nystagmus, der vermutlich durch Endolymphbewegung veranlasst wird.

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THE EFFECT OF BLOCKAGE OF ALL SIX SEMICIRCULAR CANAL DUCTS ON NYSTAGMUS PRODUCED BY DYNAMIC LINEAR ACCELERATION IN THE CAT[1]

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Seven cats were given rotary tests about an earth-vertical axis and also about an earth-horizontal axis. They were tested with the sagittal head plane in the plane of rotation as well as with the horizontal head plane in the plane of rotation. Recordings of eye movements were made during the tests. Following surgical transection and blocking of all six semicircular canal ducts, the cats were tested again. It was found postoperatively that horizontal and vertical nystagmus in response to angular acceleration about an earth-vertical axis were *abolished*. Nystagmus during constant velocity rotation about an earth-horizontal axis *remained* however although the slow phase eye velocity and frequency of the nystagmus were *reduced*. *From these and other data it is suggested* that although continuity of the semicircular canal ducts is necessary for the nystagmus in response to angular accelerations about vertical axis, it is only contributory (and not necessary) for the nystagmus during constant velocity rotation about an earth-horizontal axis. *These findings and conclusions* are discussed in the context of existing data and theory

The sensory system which causes nystagmus during exposure to changing linear acceleration has been the subject of considerable interest and speculation (Benson & Bodin 1966 Correia & Guedry 1966 de Nó, 1931 Guedry 1965 Janeke, 1968 Janeke & Jongkees, 1968 Jongkees & Philipszoon, 1962 McCabe, 1964 Niven *et al.*, 1966)

Several investigators (Guedry 1965 Janeke 1968 Janeke & Jongkees, 1968 Jongkees & Philipszoon, 1962 McCabe, 1964) have suggested that changing linear acceleration stimulates the otoliths and produces nystagmus. Other investigators (de Nó, 1931 Benson & Bodin, 1966 Benson *et al* 1967) have suggested that stimulation of the ampullary receptors causes the nystagmus.

De Nó (1931) suggested that movement of the membranous semicircular canal relative to the bony labyrinth causes relative cupular deflection and nystagmus. Benson & Bodin (1966) have speculated that the semicircular canals undergo a change in shape when gravity is coplanar with the canal and this deformation *during rotation of the canal causes endolymph* to move around the arm of the canal with consequent stimulation of the crista. Janeke (1968) has reportedly sectioned the utricular nerve bilaterally in rabbits, he reported that the "horizontal-axis nystagmus" was abolished postoperatively and he concluded that this nystagmus originates from the otoliths.

The present investigation represents another avenue of approach to selective ablation of the components of the labyrinth, and an evaluation of their individual responses to the dynamic linear acceleration produced by horizontal axis rotation

[1] DREТ Report no. 728.

METHODS[1]

Seven cats were given rotary tests and otolith tests, and were then subjected to a bilateral operation in which the ducts of all six semicircular canals were plugged. Ten days post operatively the tests were repeated. Serial histological sections of the temporal bones of all seven cats were made later using the Igarashi (1966) procedures, in order to confirm that the operations accomplished their objectives without unintended damage to ampullae or otoliths.

Subjects

Seven cats were selected for this experiment on the basis of the following criteria. (i) production of a continuous unidirectional horizontal nystagmus in response to rotation about an earth-horizontal axis at 10 rpm (60 /sec), (ii) a clear postrotary horizontal and vertical nystagmus in response to deceleration from 10 rpm (60 /sec) in 5 sec following vertical axis rotation, and (iii) absence of spontaneous and positional nystagmus prior to and following surgical deactivation of all six semicircular ca-



Apparatus

The rotary device used permits rotation about either an earth-vertical or an earth-horizontal axis. By use of a gimbal system on the vertical axis, and by selective positioning on the horizontal axis, an animal may be rotated with the head on the axis of rotation to produce either horizontal or vertical nystagmus. The device has the capability for controlled clockwise (CW) or counter clockwise (CCW) rotation at constant velocity up to 40 rpm. The experimental animals were secured to the device by a box fitted with a head holder which is a modification of the one described by Henriksson *et al* (1961) Instead of immobilizing the animals by a wire through the canine teeth, the second and third premolars on each side of the upper jaw were drilled and two wires strung through and stretched. This modification, coupled with taping the animal's limbs to its sides, provided adequate immobilization during horizontal axis rotation.

Vertical and horizontal eye movements were recorded from subcutaneous needle electrodes placed in the appropriate positions the signals were preamplified with a Fryer & Deboo (1965) amplifier and recorded on a Beckman dynograph in an adjacent room. The system time constant was of the order of 10 sec.

Animal head position relative to earth-vertical during horizontal axis rotation, and also rotation velocity were recorded from three photo cells mounted orthogonally on the rotary platform.

Surgery

A complete description of the surgical procedure used in this experiment is presented elsewhere (Money 1961) Essentially the procedure consists of transection of the arm of the semicircular canal by drilling across it with a diamond burr This causes bone chips to be packed into the two open ends produced by the transection and results in discrete plugging of the bony and membranous semicircular canal ducts, so that endolymph movement around the arm of the canal is not possible postoperatively The cochlea and otoliths, and even the ampulla of a canal plugged in this way remain morphologically normal (Money 1967) This technique may be used selectively to inactivate any single semicircular canal or all of them as in this experiment.

Nomenclature

To facilitate discussion of the rotary configurations used in this experiment a generalization of the man-referenced kinematic acceleration nomenclature presented elsewhere (Hixson *et al* 1966) will be used here.

A sketch of a cat's head showing the cardinal head axes and linear and angular accelerations with respective polarities is presented in

[1] The principles of laboratory animal care as specified by the American Physiological Society were observed during the course of this experiment

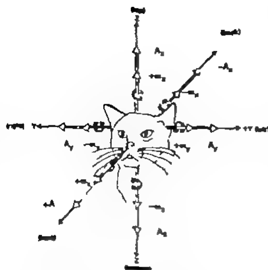


Fig. 1. Sketch of cat's head showing direction and polarity of cardinal head axes and linear and angular accelerations, based on kinematics nomenclature.

Fig. 1. As may be seen in Fig. 1, the x, y and z head axes denote the front-back, left-right, and top-bottom axes of the head. The corresponding frontal, sagittal, and horizontal head planes are denoted as yz, xz and xy respectively. Angular acceleration about any of these head axes is denoted by the angular acceleration vector $\bar{\alpha}$, with a subscript to indicate the axis along which the vector is aligned. For example, α_x, α_y and α_z denote roll, pitch and yaw accelerations. The polarity of the angular acceleration is determined from Fig. 1 by a right hand rule, i.e., when the fingers of the right hand are curled about the rotational axis in the direction of the angular acceleration, the acceleration is described by a vector drawn in the direction denoted by the thumb. For example $+\alpha_z$ denotes counterclockwise (CCW) yaw acceleration (as seen from above) and clockwise (CW) yaw deceleration; $-\alpha_z$ denotes CW yaw acceleration and CCW yaw deceleration.

Linear acceleration is also denoted by a vector $\bar{A}$, with a subscript to indicate the axis along which it acts or a double subscript to indicate the plane in which it acts. The direction of the vector $\bar{A}$ in a given plane is specified by the angle ϕ with a subscript to indicate the axis about which the vector rotates. For example, when a cat is rotated head over tail about an earth-horizontal axis (as in one of the conditions of the present experiment) the vector $\bar{A}_{xz}$ representing gravitational acceleration (g) rotates in the sagittal (xz) head plane. This may be indicated by a magnitude/direction notation as $\bar{A}_{xz} = g \angle \phi_y$, in which $g = 32$ ft sec^2 and ϕ_y is the displacement of the vector from $\phi_y = 0$ (vector directed along the $+$ axis). Similarly, when a cat is rotated about an earth-horizontal axis so that the linear acceleration due to gravity acts in the xy head plane (as in another of the conditions of the present experiment), the gravitational vector is denoted by $\bar{A}_{xy} = g \angle \phi_z$ where $\phi_z = 0$ when the vector is directed along the $+x$ head axis.

Vestibular tests

The experimental sequence, both preoperatively and 10 days postoperatively, was as follows: otolith tests; optokinetic stimulation (OKS-I); earth-vertical axis stimulation; optokinetic stimulation (OKS-II); earth-horizontal axis stimulation; optokinetic stimulation (OKS-III).

The otolith function of the cats was assessed by tests described previously (Money & Scott, 1962). These tests, which were done with the animal blindfolded, included slow angular displacements, linear accelerations, and "drop" tests.

The optokinetic stimulations were carried out for the purpose of calibrating the eye movement records. Each of the three optokinetic stimulations took place in a lighted room and consisted of four 75-second rotations about an earth-vertical axis at a constant velocity of 5 rpm (30°/sec). The four rotations included two (CW and CCW) about the cat's z axis, to produce horizontal nystagmus, and two (CW and CCW) about the cat's y axis, to produce vertical nystagmus.

Vertical axis stimulation consisted of eight rotary profiles, four about the cat's z axis and four about the y axis. For each z axis and

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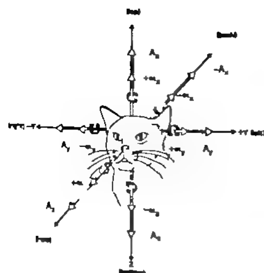


Fig. 1 Sketch of cat's head showing direction and polarity of cardinal head axes and linear and angular accelerations, based on kinematics nomenclature.

Fig. 1 As may be seen in Fig. 1 the x y and z head axes denote the front back left-right, and top-bottom axes of the head. The corresponding frontal, sagittal, and horizontal head planes are denoted as yz xz and xy respectively Angular acceleration about any of these head axes is denoted by the angular acceleration vector $\bar{\alpha}$, with a subscript to indicate the axis along which the vector is aligned For example, α_x, α_y and α_z denote roll, pitch and yaw accelerations. The polarity of the angular acceleration is determined from Fig. 1 by a right hand rule, i.e., when the fingers of the right hand are curled about the rotational axis in the direction of the angular acceleration, the acceleration is described by a vector drawn in the direction denoted by the thumb. For example $+\alpha_z$ denotes counter clockwise (CCW) yaw acceleration (as seen from above) and clockwise (CW) yaw deceleration, $-\alpha_z$ denotes CW yaw acceleration and CCW yaw deceleration.

Linear acceleration is also denoted by a vector A, with a subscript to indicate the axis along which it acts or a double subscript to indicate the plane in which it acts. The direction of the vector $\bar{A}$ in a given plane is speci-

fi ... script to ... or rotat ... ex ... tated head ov ... about ... axis (as in cr ... th ... ent experimen ... vec ... A ... ravitational acc ... tion ... rotat ... ittal (xz) head p ... This may be m ... a magnitude dire ... tion rotation as. A ... in which $g = 32$ ft/sec and ϕ_y is the displacement of the vector from ... 0 (vector directed along the + axis) Similarly when a cat is rotated about an earth-horizontal axis so that the linear acceleration due to gravity acts in the xy head plane (as in another of the conditions of the present experiment) the gravitational vector is denoted by $\bar{A}_{xy} = g \angle \phi_z$ where $\phi_z = 0$ when the vector is directed along the $+x$ head axis.

Vestibular tests

The experimental sequence, both preoperatively and 10 days postoperatively was as follows. otolith tests optokinetic stimulation (OKS-I) earth-vertical axis stimulation optokinetic stimulation (OKS-II) earth-horizontal axis stimulation optokinetic stimulation (OKS-III)

The otolith function of the cats was assessed by tests described previously (Money & Scott, 1962) These tests, which were done with the animal blindfolded, included slow angular displacements, linear accelerations, and drop tests.

The optokinetic stimulations were carried out for the purpose of calibrating the eye movement records. Each of the three optokinetic stimulations took place in a lighted room and consisted of four 75-second rotations about an earth-vertical axis at a constant velocity of 5 rpm (30 /sec) The four rotations included two (CW and CCW) about the cat's axis, to produce horizontal nystagmus, and two (CW and CCW) about the cat's y axis, to produce vertical nystagmus.

Vertical axis stimulation consisted of eight rotary profiles, four about the cat's axis and four about the y axis. For each axis

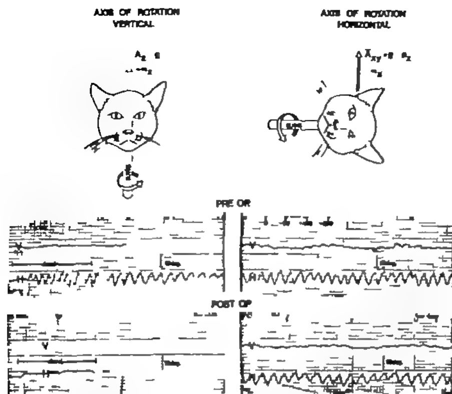


Fig 2 Horizontal (*H*) and vertical (*V*) eye move nts prior to (*Pre op*) and following (*Post op*) block of endolymph flow in all six semicircular canals. left traces show response to deceleration (in) from 120°/sec CW rotation about an earth-rtical axis. Two right traces show response to constant velocity CCW rotation about an earth-horizontal axis at 60°/sec. The angular acceleration (α) during vertical axis rotation and the linear acceleration ($\bar{A}_{xy}$) during horizontal axis rotation act in the horizontal head plane. The horizontal nystagmus has fast component toward the cat s left.

y axis orientation the animal received CW and CCW rotations at 10 rpm (60 /sec) and 20 rpm (120 /sec) Each rotary profile consisted of acceleration to constant angular velocity maintenance of this velocity for 120 seconds and deceleration to zero velocity in 5 sec, which resulted in an average deceleration of 12 /sec² from 10 rpm and 24 /sec² from 20 rpm. Prior to vertical axis rotation, eye movements were recorded in the dark for 45 sec to test for spontaneous nystagmus.

The eight profiles for horizontal axis stimulation were the same as for vertical axis stimulation. Rotations were carried out in the dark with careful attention given to the elimination of light leaks. Chatter and noise were created during rotation in an attempt to keep the animal aroused" A one minute rest period was given between successive rotations. Prior to horizontal axis rotation, eye movements were recorded for 45 sec with the animal's nose successively up right, left, and down, to test for positional nystagmus.

RESULTS

Preoperatively the otolith function of the cats was judged normal (Money & Scott, 1962) except in cat 259 (a white cat) which was assessed as otolith defective No clear change in otolith function was observed in any cat post operatively

Eye movement tracings illustrating the preoperative (Pre op) and postoperative (Post op) responses produced by the rotary stimuli are presented in Fig. 2 and 3 As indicated by the sketches and symbols above the tracings, angu-





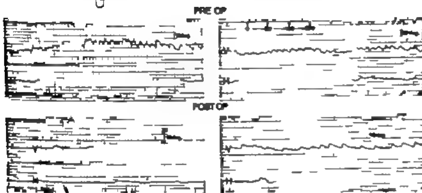


Fig 3 Horizontal (*H*) and vertical (*V*) eye movements prior to (*Pre op*) and following (*Post op*) blockage of endolymph flow in all six semicircular canals. Two left traces show response to deceleration (in 5 sec) from 120°/sec CW rotation about an earth-vertical axis. Two right traces show response to CCW rotation about an earth-horizontal axis at 60°/sec. The angular acceleration (α_y) during vertical axis rotation and the linear acceleration (A_{xz}) during horizontal axis rotation act in the sagittal head plane. The vertical nystagmus has fast component toward the lower lid.

lar acceleration during vertical axis rotation and linear acceleration during constant velocity horizontal axis rotation acted in the horizontal (xy) head plane for the results displayed in Fig. 2, and in the sagittal (xz) head plane for the results displayed in Fig. 3 In both figures it may be observed that the nystagmus (horizontal nystagmus in Fig. 2, and vertical nystagmus in Fig. 3) produced preoperatively by vertical axis deceleration (CW decel from 120 /sec in 5 sec) is *abolished* following "plugging" of all six semicircular canals the response to rotation at constant velocity about an earth-horizontal axis (CCW 60 /sec) *remains*

Fig. 4 summarizes the eye movement responses for all animals observed during exposure to *horizontal axis rotation* prior to (Pre op) and following (Post op) canal plugging Mean instantaneous eye velocity of slow phase horizontal nystagmus is shown plotted at 30 intervals during 360 (1 revolution) rotation of the linear acceleration vector $\bar{A}_{xy} = g \angle \phi$ at rates of 60 /sec (Fig. 4 *a*) and 120 /sec (Fig. 4 *b*) Each point represents the mean of 28 values [7 animals × 4 revolutions × 1 direction of rotation] Presented in Fig. 4 *c* is mean instantaneous slow phase eye velocity of vertical nystagmus for 60 /sec constant rotation of the linear acceleration vector $\bar{A}_{xz} = g \angle \phi_y$. Each point represents the mean of 28 values [7 animals × 4 revolutions × 1 direction of rotation (CCW)]. Also presented in each plot is an estimate of the number of nystagmic beats occurring during one revolution. This estimate is expressed as beats/cycle for both Pre op and Post op tests.

No plot of vertical nystagmus in

a

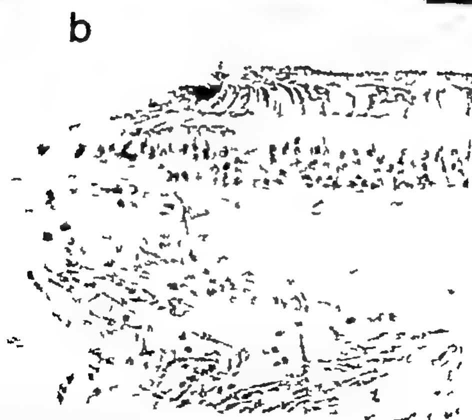


Fig 6 High magnification of: ( ) the crist f the horizontal semicircular canal, cat 31 (370) (*b*) the utricular otolith organ, cat 312 (400)

same phase relationship to the direction of the $\bar{A}_{xy}$ vector relative to the head. In both cases the maximum magnitude of the slow phase velocity occurs near 0 (nose up) and the minimum occurs near 180 (nose down) This cyclic variation of velocity has been observed previously in humans, also at 60 /sec (Benson & Bodin 1966 Correia & Guedry 1966) and its origin has been questioned. The present data suggest that, in the case of the cat, the oscillation is produced not by a modulating influence of a response of the semicircular canals to endolymph flow around the canal nor by a displacement of the membranous canals relative to the bony canals, but by some complementary system such as the otoliths

The suggestion that complementary systems may act during horizontal axis rotation has been made previously Correia & Guedry (1966) speculated that both the semicircular canals and otolith organs act in concert during horizontal axis rotation (Correia & Guedry 1966 p 307) Young (1967) has proposed a model in which otolithic and semicircular canal subsystems contribute to the total nystagmic output in response to dynamic linear acceleration At first sight the suggestion that two operant systems respond to horizontal axis rotation appears in direct conflict with the data of Janeke (1968) who found that sectioning the utricular nerves in rabbits abolished the response to horizontal axis rotation. Assuming that the utricular nerve sections were discrete, one could conclude that the otoliths are necessary for the initiation and maintenance of the nystagmus as well as the cyclic modulation of eye velocity whereas the canals contribute to the nystagmus frequency and slow phase velocity

The foregoing discussion has been concerned with the horizontal nystagmus observed during rotation of the linear gravitational vector in the *xy* (horizontal) head plane. The vertical nystagmus produced by rotation of this vector in the *xz* (sagittal) head plane was different in several ways: (1) a greater decrease in slow phase eye velocity (slow phase up) occurred following canal plugging (Fig. 4 *c* vs. 4 *a*) (2) consistent up beating (slow phase down) vertical nystagmus was absent both preoperatively and postoperatively (3) consistent up beating or down beating vertical nystagmus was absent at 120 /sec rotational velocity These differences between horizontal and vertical nystagmus suggest different response characteristics for the sensory systems associated with the two planes of stimulation Apparently no published data exist for humans exposed to horizontal axis rotation so that the g" vector rotates in the sagittal head plane Niven *et al.* (1966) however oscillating humans on a track such that the resultant linear acceleration vector swung ±30 in the sagittal head plane, observed no vertical nystagmus although the same stimulus in the horizontal head plane produced horizontal nystagmus. The presence of more down beating than up beating vertical nystagmus in response to horizontal axis rotation observed in the present study has been reported previously for cats (Money & Scott, 1962) during angular acceleration about an earth-vertical axis It is beyond the scope of the present study to speculate upon the origin of this directional disparity but it should be noted that in the present study it was also observed for vertical *optokinetic* nystagmus.

The membranous canals were "tacked" to the bony labyrinth by the operation.

ACKNOWLEDGMENTS

Technical assistance was provided by Mr Dale Cinnamon and Mr A. Nicholas. Mr Jerry Laufer prepared the histological sections. Photography was by Mr Fred Wilson. The rotator was built by the Technical Services Section of DRET and the preamplifiers were built by the Electronics Section f DRET

ZUSAMMENFASSUNG

Drehversuche wurden an sieben Katzen infolgender Weise ausgeführt. Sie wurden teils um Ine vertikale und teils um eine horizontale Achse rotiert. Die Tiere wurden in beiden Versuchsserien teils mit der sagittalen Kopf bene parallel zur Rotationsebene und teils mit der horizontalen Kopfebene in der Rotations-

ebene gedreht. Die Augenbewegungen wurden während der Untersuchung elektrisch registriert.

Nach chirurgischer Durchschneidung und Plombierung aller sechs halbzirkelförmigen Kanäle wurden die Vestibularisfunktionen der Katzen wieder in der selben Weise untersucht. Postoperativ konnte dann kein Nystagmus, weder horizontaler noch vertikaler als Resultat der Winkelbeschleunigung um eine vertikale Rotationsachse ausgelöst werden. Die Rotation mit konstanter Rotations-geschwindigkeit um eine horizontale Achse gab postoperativ noch immer den selben Nystagmus, obwohl die Geschwindigkeit der langsamen Phase und die Frequenz der Nystagmusschläge reduziert waren.

Die Resultate dieser und anderer Untersuchungen haben zu der Schlussfolgerung geführt, dass eine normale Funktion der halbzirkelförmigen Kanäle unentbehrlich ist für die Auslösung eines Nystagmus als Folge Winkelbeschleunigungen um eine vertikale Achse Zur Auslösung des Nystagmus durch Rotation um eine horizontale Achse mit konstanter Rotationsgeschwindigkeit kann die Kanalfunktionen höchstens eine beitragende Wirkung haben. Diese Befunde und Schlussfolgerungen wurden diskutiert.

Addendum In addition to the cats whose data are included in the present experiment, several cats whose canals had been plugged for longer periods of time were also tested. Among these were cats 68 and 212. Preliminary observations by Money indicated that cats 68 and 12 showed no horizontal nystagmus during a counterrotation stimulus where the component of the linear acceleration acting in the horizontal head plane was 0.25 g and rotated in the head plane at 120°/sec (in discussion of paper by F E. Guedry Influence of linear and angular accelerations on nystagmus. *Second Symposium on The Role of the Vestibular Organs in Space Exploration.* NASA SP 115 pp. 185–199 1966). These same two cats when exposed to horizontal axis rotation (1 g, 60°/sec), such as used in the present experiment, exhibited a continuous unidirectional horizontal nystagmus.

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THE INTERACTION BETWEEN THE UTRICLE AND THE SACCULE

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By using a combination of series of investigations on human subjects in the human centrifuge and neurophysiological experiments on the cat it has been possible to solve the problem of the influence of the saccule and the utricle on the oculomotor system, and the cooperation between the two organs during linear accelerations on different planes.

Linear acceleration, including gravity is considered to be the effective stimulus of the otoliths. Various theories as to the manner in which these organs are stimulated have been advanced (Breuer 1875 de Kleyn, 1923 Quix, 1925) However none of them can explain all the known facts.

Three different theories about the saccular function have been developed. Several authors have shown that the saccule reacts to linear acceleration whereas others deny that this organ exerts any vestibular function. Other authors again have shown that the saccule reacts to sound, indicating that it is a cochlear organ. (For references see Fluur & Mellström, 1970.)

Concerning the utricle, all authors seem to agree that it is an organ reacting to gravity and linear acceleration. The theory put forward by Breuer (1891), that the adequate stimulus of the utricle is the tangential force parallel to the surface has been proved by Steinhausen (1935) and by von Holst (1950)

The influence of the otolith organs on the oculomotor system has been studied by several authors, but their methods of investigation have generally implied a mass stimulation of the whole organ and, consequently the experiments have usually resulted only in theories. Quite recently Fluur & Mellström (1970) have been able to show through electrical stimulation of different areas on the utricular and saccular surface on the cat, that distinct eye movements can be released from these areas The results can be divided in two groups in one group alert animals made coordinated eye movements, and in the other sleeping animals made uncoordinated movements. From the latter group one can conclude which eye muscles are stimulated from respective areas.

The superior saccular area produced coordinated elevation in alert cats, and in sleeping cats, uncoordinated eye movements, mainly the activation of the ipsilateral superior rectus and the contralateral inferior oblique From the inferior saccular area a coordinated depression was observed in alert animals, and in sleeping cats uncoordinated eye movements were obtained, the principal contraction being that of the ipsilateral superior oblique and of the contralateral inferior rectus (Fig. 1)

Stimulation of the utricle gave the following results. From the anterior medial (*AM*) and the posterior lateral areas (*PL*) a coordinated elevation was obtained in alert animals from the anterior lateral (*AL*) and the posterior medial areas (*PM*) a coordinated depression was observed. Furthermore, from the midlateral area (*ML*) there was a horizontal eye movement in contralateral direction (Fig. 2) In sleeping animals, however the phenomena were more complex. *AM* gave a contraction

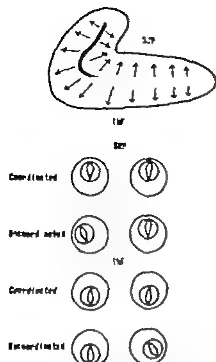


Fig 1 Coordinated and uncoordinated eye movements obtained by electrical stimulation of the left superior and inferior saccular areas respectively

of the ipsilateral superior rectus and the contralateral inferior oblique, whereas *PL* activated the contralateral superior rectus and the ipsilateral inferior oblique. From *AL* the ipsilateral inferior rectus and the contralateral superior oblique were activated, while the contralateral inferior rectus and the ipsilateral superior oblique were stimulated from *PM* The area *ML* gave the same responses as in alert animals (Fig. 3)

The incitement to undertake the above-mentioned neurophysiological studies was derived from a series of investigations on human subjects in the human centrifuge (Brandt & Fluur 1966 1967 *a b c*) The results of these investigations gave birth to a theory as to the influence of the otoliths on the eye movements. This theory could not be proved, however by human experiments, but has now been confirmed through electrophysiological animal investigations (Fluur & Mellström, 1970) Thanks to these combined animal and human experiments it is now possible to explain both how the utricle and the saccule function individually and how they cooperate with each other In this paper only the latter aspect will be elucidated.

If an individual is exposed to linear acceleration straight forward or is centrifuged with the chair erect and his face toward the centre, the eyes are subjected to a vertical downward-directed deviation (Brandt & Fluur 1967 *b*) Fig. 4 shows the amount of eye deviation in relation to the actual direction of the resultant vector There is almost an exponential increase in the size of the deviation for every increase of Φ by 10 The deviation is especially great between points 6 and 7 (50 and 60 resp) Subsequently the amount of deviation decreases in spite of further increase in Φ and

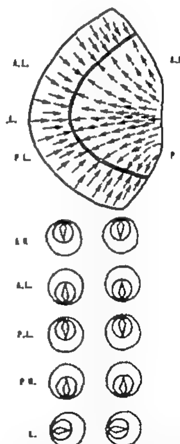


Fig 2 Coordinated eye movements obtained by electrical stimulation of different areas on the left utricle, *AM* anterior medial area, *AL*, anterior lateral area, *ML*, mid-lateral area, *PL*, posterior lateral area, *PM* posterior medial area.

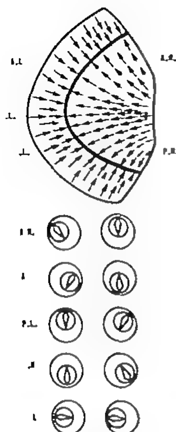


Fig 3 Uncoordinated ey movements obtained by electrical stimulation of different areas on the left utricle. Abbreviations are the same as in Fig. 2.

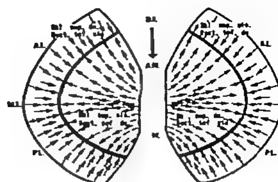


Fig 5 Stimulated (white) and inhibited (vertically striped) areas on the utricular surfaces during linear acceleration forwards. The activated eye muscles ar shown. Abbreviations are the same as in Fig. ..

in the centrifugal force. This depression of the eyes is induced by stimulation partly of *AL* and *PM* on the utricular surface (Fig. 5) and partly of the inferior saccular area (Fig. 6) The decreasing depression, in spite of further increase of stimulus, is due to the fact that the resultant force starts to influence the superior saccular area, which tries to inhibit the depression of the eyes This is still more clearly seen if the same stimulus is applied, but with this difference, that the subject tilts his head 50 backwards (Fig. 7) A vertical downward-

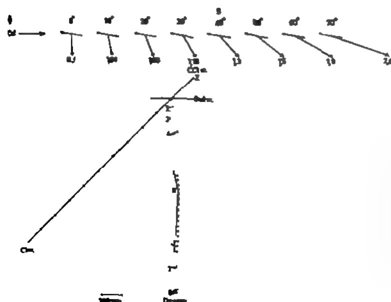


Fig 4 The amount of eye deviation in relation t the actual direction of the resultant vector D.f direction of force R.f resultant force in g X Y Z, the three axes of eye movement. The almost horizontal arrows bove the curve indicate the position of the head (seen from the side) in relation to D.f and the vertical" arrows below it show the of R.f and its directi

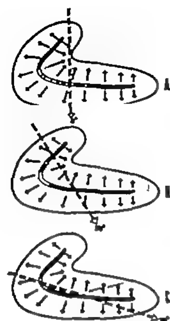


Fig 6 Stimulated (white) and inhibited (vertically striped) areas on the left saccular surface in normal position. The large dotted arrows indicate direction and size of the resultant force.

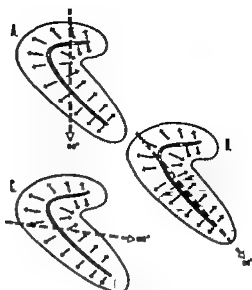


Fig 8 Stimulated (white) and inhibited (vertically striped) areas on the left saccular surface, with the subject tilted 50° backwards. The horizontally striped areas indicate spontaneous activity The large dotted arrows indicate direction and size of the resultant force.

directed eye movement is obtained here also, but differs from the earlier situation inasmuch as here there is an almost exponential decrease in the deviation up to point 4 (80) where after the direction of the deviation suddenly changes and the eyes begin to move upwards despite further increase in the force of stimulation In this case the influence of the resultant force on the superior saccular area will become more and more pronounced with increasing force of stimulation, even to the extent of totally inhibiting the inferior saccular area and, consequently also the downward-directed eye movement (Fig. 8) From animal experiments we also know that it is easier to obtain elevation than depression of the eyes during stimulation of the otoliths (Fluur & Mellström, 1970) The conclusion is, that there is a reciprocal relation between those areas on the utricular surface (*AL* and *PM*) which result in a depression and the superior saccular area which causes an elevation.

If a subject is exposed to linear acceleration backwards, or is centrifuged with his face directed from the centre, the eyes move vertically upwards (Brandt & Fluur 1967 *c*) (Fig. 9).

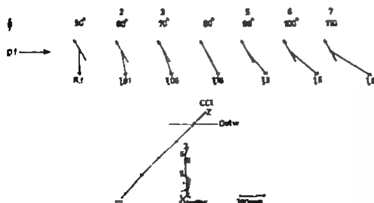


Fig 7 The amount of eye deviations in relation to the actual direction of the resultant vector Φ during linear acceleration forwards, with the subject tilted 50° backwards. The explanations are the same as in Fig. 4.

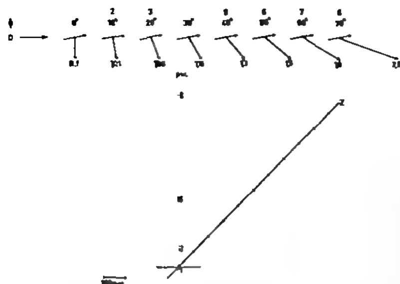


Fig. 9 The amount of eye deviation in relation to the actual direction of the resultant vector Φ during linear acceleration backwards, with chair erect and the head in normal position. The explanations are the same as in Fig. 4

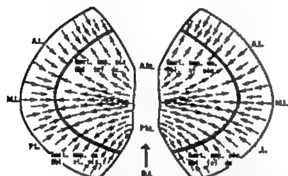


Fig. 10 Stimulated (white) and inhibited (vertically striped) areas on the utricular surface during linear acceleration backwards. The activated eye muscles are shown. Abbreviations are the same as in Fig. 2.

The amount of deviation is, however considerably smaller than during acceleration forwards. During acceleration backwards the utricular areas *AM* and *PL* are stimulated (Fig. 10), and simultaneously the inferior saccular area is also stimulated, which, under normal conditions is always influenced by 1 *g*. Here again we have two antagonistic areas the utricular *AM* and *PL*, which are elevating the eyes, and the inferior saccular area, which tries to cause a depression. The fact that the amount of the deviation is considerably smaller than during acceleration forwards is due to just this inhibiting effect on the elevation of the inferior saccular area.

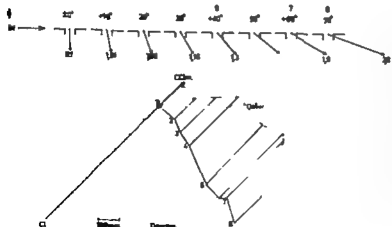


Fig. 11 The amount of eye deviation in relation to the actual direction of the resultant vector Φ during linear acceleration with the force acting in the frontal plane, and with the head in normal position. The size f the diagonal lines on the curve indicates the size of ocular rotation. The symbols bove the curve indicate the head with the utricles and saccules seen from behind. The vertical arrows indicate the size of *R.f* and its direction. Abbreviations are the same as in Fig. 4.

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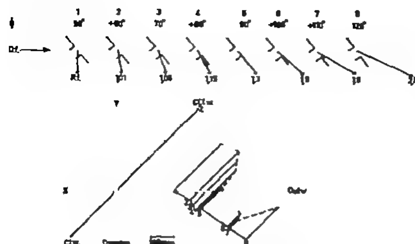


Fig 12 The amount of the eye deviations in relation to the actual direction of the resultant vector Φ during linear acceleration, with the force acting in frontal plane, and with the subject's head tilted 50° outwards from the centre. Symbols and abbreviations are the same as in Fig. 11

We have seen that linear acceleration forwards and backwards result in vertical eye movements (*Y* axis) but no reactions along the other two axes (*X* and *Z*) If, on the other hand, a person is exposed to linear acceleration with the force acting in the frontal plane, simultaneous eye movements are observed along all the three axes of rotation (Brandt & Fluur 1967 *a*) If the subject is centrifuged with his head in normal position, with the centre of the centrifuge to the left, and the face in the direction of rotation, he is subjected partly to a horizontal eye deviation to the right (*X* axis) partly to a depression (*Y* axis) and partly to a counterclockwise rotation (*Z* axis) (seen from the subject) (Fig 11) The movements along the X and Y axes increase almost uniformly during the whole stimulation, whereas the rotatory eye movement (*Z* axis) at first increases greatly but subsequently again decreases despite the increasing resultant force. The reason for this is more readily understood if the same experiment is made with the subject's head tilted 50 outwards from the centre (Fig. 12) The angle of the resultant force is then increased from 50 and upwards, so that the force is soon parallel with the utricular surface, and perpendicular to the left saccular surface. At that moment, the rotatory eye movement suddenly diminishes. This depends on the fact that the utricular combination $AL+PL$ sin. and $AM+PM$ dx. (Fig. 13) are together antagonists to the combined effect from the superior and the inferior areas of the left saccule (Fig. 14) Stimulation of the utricular areas gives rise namely to counterclockwise rotation, while, on the contrary stimulation of the whole left saccule results in clockwise rota-

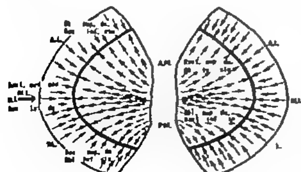


Fig 13 Stimulated (white) and inhibited (vertically striped) areas on the utricular surfaces during linear acceleration with the force acting in the frontal plane. The activated eye muscles are shown. Abbreviations are the same as in Fig. 2.

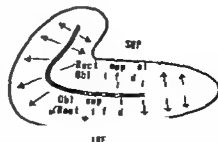


Fig 14 The stimulated left superior and inferior saccular areas during linear acceleration with the force acting in the frontal plane. The activated eye muscles are shown.

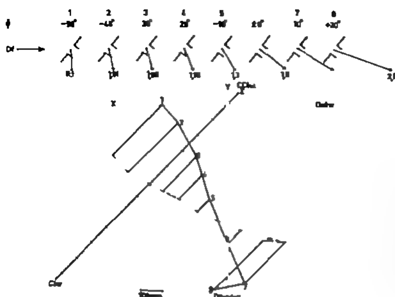


Fig. 15. The amount of eye deviation in relation to the actual direction of the resultant vector Φ during linear acceleration, with the force acting in the frontal plane, and with the subject's head tilted 50° inwards toward the centre. *Symbols and abbreviations are the same as in Fig. 11*

tion. When the resultant angle is perpendicular to the left saccule, this organ inhibits the rotatory eye movement induced by the utricle, which is also clearly shown in the curves (Fig. 12).

Concerning the two eye movements along *X* and *Y* axes, the horizontal movement is released from the utricular *ML* area, while the vertical movements are obtained from the inferior saccular areas on both sides. During stimulation in the frontal plane the utricle mostly functions in producing rotatory eye movements. This statement is proved by Fig. 15, where the subject is exposed to centrifugation with the force acting in the frontal plane, and with the head tilted 50° toward the centre. At first a clockwise rotation of the eyes is obtained, but this decreases to 0° when the resultant angle is perpendicular to the utricular surface. Finally, when the resultant force has passed the "zero point" the rotation of the eyes changes to the opposite direction.

CONCLUSIONS

Sagittal stimulation

A. Utricular areas, where the sensory cells are orientated backwards, are stimulated during linear acceleration forwards, and cause vertical eye movements downwards.

B. Utricular areas, where the sensory cells are orientated forwards, are stimulated during linear acceleration backwards, and cause vertical eye movements upwards.

C. The inferior and anterior saccular area, where the sensory cells are orientated downwards and forwards, is stimulated during linear acceleration upwards and backwards, and gives rise to vertical eye movements downwards.

D. The superior saccular area, where the sensory cells are orientated upwards and backwards, is stimulated during linear acceleration downwards and forwards, and results in vertical eye movements upwards.

E. Synergism exists between points *A* and *C* and between points *B* and *D*.

F. Antagonism exists between points *A* and *B* and between points *A* and *D*.

Frontal stimulation

G. Utricular areas, where the sensory cells are orientated to the right, are stimulated during linear acceleration in the frontal plane to the left, and cause partly horizontal eye movements to the right and partly counterclockwise rotation.

H Utricular areas, where the sensory cells are orientated to the left, are stimulated during linear acceleration in the frontal plane to the right, and give rise to partly horizontal eye movements to the left and partly clockwise rotation.

I The saccule can give rise to rotatory eye movements only if the stimulus is perpendicular to its surface, when both the two areas are stimulated simultaneously consequently within a very narrow space The left saccule then functions antagonistically to those sensory cells on the left utricular surface which react according to point *G* and the right saccule functions antagonistically to those reacting according to point *H*

ZUSAMMENFASSUNG

Durch Gebrauch einer Kombination von Untersuchungen an Menschen in der Menschenzentrifuge und neurophysiologischen Experimenten an Katzen ist es möglich geworden das Problem des Einflusses von Sacculus und Utriculus auf das oculomotorische System und die Zusammenarbeit der zwei Organen durch lineare Beschleunigung in verschiedenen Ebenen zu lösen.

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INTERACTION BETWEEN STATOLITH ORGANS AND SEMICIRCULAR CANALS ON APPARENT VERTICAL AND NYSTAGMUS

Investigations on the effectiveness of the statolith organs

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The effect of postrotatory vertical canal stimulation on nystagmus and apparent vertical was investigated as function of the tilt position at stop in a vertical roll-plane. Nystagmus duration and number of beats as well as the ffect on the apparent vertical increased with change of stop position from head-up to head-down. The results are interpreted in terms of hypothesis which proposes decline of effectiveness of the statolith organs with increasing degree f tilt. This is discussed with respect to related findings.

Responses such as *nystagmus* due to the stimulation of the semicircular canals, can be modified by a concomitant linear acceleration. In man, constant rotation in yaw about a horizontal axis (i.e in a vertical rotation plane) caused a unidirectional fluctuating nystagmus which lasted as long as rotation continued. After cessation, the nystagmus and also the subjective phenomena were much shorter than those after *yaw-rotation in the horizontal plane* (Guedry 1965 Benson & Bodin, 1966 *a*) Because these findings could not be explained by a direct effect of gravity on the cupula (Correia & Guedry 1964 Guedry 1965 Benson & Bodin, 1966 *a*) the following hypothesis was proposed by Guedry During rotation in a vertical plane the responses to canal stimulation are enhanced by the statolith organs which are stimulated by the continuously re-orienting linear force. After cessation, however there is a contradiction between the information from canal- and gravireceptors. The canal-receptors signal rotation, that is, change of position, whereas the statolith organs signal a fixed position. This discrepancy causes the suppression of the nystagmus. Benson & Bodin (1966 *a*) criticized this point of view They proposed, on the basis of different specific densities of endolymph and perilymph, a direct effect of gravity on the canals. that the component of gravity which is co-planar to the stimulated canals would be effective in cupula restoration

As far as the *perrotatory* responses are concerned Janeke (1968) investigated partial labyrinthectomized rabbits the canal system was left intact, the saccular maculae and the utricular nerves were *bilaterally destroyed*. During vertical plane rotation about a sagittal (roll-) axis he observed only a transitory nystagmus, which indicated the intactness of the canal system. The sustained unidirectional nystagmus, however had ceased completely This indicates that the continuous perrotatory nystagmus originates in the statolith organs as was suggested by Guedry (1965)

Concerning the *postrotatory* responses a critical behavioural experiment with humans was performed by Benson (1966), the results

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of which also favoured the hypothesis of Guedry

This hypothesis implies that the degree of contradiction between the signals from canal- and gravireceptors increases as the (subject fixed) plane of rotation is varied from horizontal to vertical. No contradiction is caused after stop in a horizontal plane because the gravireceptors cannot differentiate between positions in this plane. In a vertical rotation plane, however gravireceptors differentiate optimally between positions, therefore the degree of contradiction has a maximum value.

Another aspect of the interaction between the statolith organs and canal receptors concerns the *effectiveness* of the statolith organs. Whereas Guedry's hypothesis postulates maximal influence of the statolith organs after stop in a vertical plane as compared with the horizontal plane, it bears no implication about the influence of the degree of tilt at stop in a vertical plane In man this effect of stop position on postrotatory nystagmus has been investigated only in the yaw-plane (Correia & Guedry 1964 Guedry 1965 Benson & Bodin, 1966 *a)* The nystagmus seemed to be slightly in the prone (nose-down) as compared the supine (nose-up) position, suggesting, that the statolith organs are more effective in suppressing the nystagmus in the prone than in the supine position. To obtain further information about the effectiveness of the statolith organs we studied postrotatory nystagmus in the roll-plane, varying the stop position over the whole range from head-up to head-down.

Thus far we have only discussed nystagmus, which is generally classified as a response to canal stimulation. Our investigations also concerned the *apparent vertical* (AV) for the perception of which the statolith organs play the principal role the relevant stimulus parameters are direction *and* magnitude of the gravito-inertial force (Schöne, 1962 Correia *et al* 1965 Miller & Graybiel, 1966 Schöne *et al.* 1967)

As to the effectiveness of the statolith organs with respect to the AV Quix & Eijsvogel (1929) were the first to notice the high degree of uncertainty of subjective orientation at the inverted positions. This and similar phenomena have been confirmed by several authors, who measured the AV variability in different ways (Fischer 1930· Brown, 1961 Schöne, 1964 Schöne & Udo de Haes, 1968 Udo de Haes, in press) including the threshold of the so-called oculogravic illusion" (Graybiel & Patterson, 1955 Graybiel & Clark, 1962) In every instance the AV was much less stable in the inverted as compared with the normal positions.

The hypothesis proposing a decline of effectiveness of the statolith organs with increasing degree of tilt was further elaborated by Schöne (1962) He related the increasing variability of the AV to the findings of von Holst & Grisebach (1951) In preliminary experiments they found that the AV is affected by postrotatory stimulation of the vertical canals in a vertical roll-plane the influence appeared to be much greater in the head-down than in the head-up position. We repeated and extended these experiments.

Also in line with the idea of a changing effectiveness of the statolith organs is the finding of Schöne & Udo de Haes (1968) that the somatoreceptors of the trunk affect the AV most strongly at the inverted positions of the head.

A theoretical analysis of this kind of interaction between sensory systems has recently been given by Bischof (1966)

In summary our investigations have three main aspects.

(1) the interaction between the signals from canal and statolith receptors on a response generally occurring with canal stimulation (nystagmus)

(2) the same interaction on a response which is mainly dependent on the stimulation of the statolith organs (apparent vertical)

(3) the decline of effectiveness of the statolith organs with increasing degree of tilt with respect to the AV and to the suppression of nystagmus.

METHOD

General

The subject (S) was placed in a bed which facilitated rotation in the roll-plane of the head (Fig. 1 *a,b*) After acceleration of 1 /sec² S was rotated with constant speed of 60 /sec for one minute his eyes were closed. He was then stopped within 1 sec at one of the following positions: 0 (head-up) 30 60, 90 120 150 and 180 (head-down) In the nystagmus series they were to the left, with an error of maximal 6 in the AV experiments they were to the right, with an accepted error of maximal 3 In both series four independent tests, two clockwise (CW) and two counterclockwise (CCW) were performed with every S.

Nystagmus

Four Ss were investigated They wore Frenzel-spectacles (Fig. 1 *a*) The fast rotatory nystagmus beats of the right eye were indicated by the experimenter and recorded on a tape Non-rotatory movements of the eye were avoided by fixation of a tiny light. The test order was randomized, with two tests daily for each S The tape records were analysed for number of beats and nystagmus duration. Also the time pattern of the beats was investigated, in order to get an estimate of both the time constant and the initial value of the slow phase velocity exponential regression analyses were per-

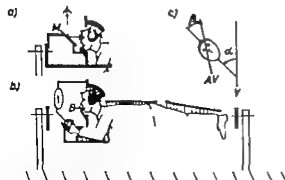


Fig. 1 Apparatus and definitions. (*a*) Nystagmus experiments; the right eye is observed with mirror (*M*). (*b*) Apparent ertical experiments, *B* bit board (*c*) Definitions: *AV* apparent ertical position of luminous line *V* vertical.

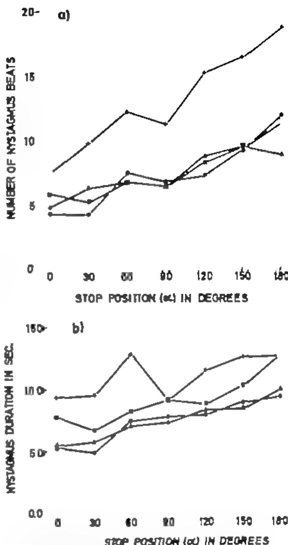


Fig 2 Nystagmus responses as function of stop position, each curve represents the data f one subject. ( ) Number of fast nystagmus beats. The mean of the values at the 0° and 30° positions differs significantly from that at the 150° and 180° positions ($p < 0.01$ for every S, Mann-Whitney U-test). (*b*) Nystagmus duration, the statistical evaluation corresponding to the above yields $p < 0.05$ for each S.

formed on the reciprocal values of the inter time between successive beats. The initial value is defined as the value at the time 2 sec after onset of deceleration (t_0)

Apparent vertical

Seven Ss, including three from the previous series, were used. After stop they kept their

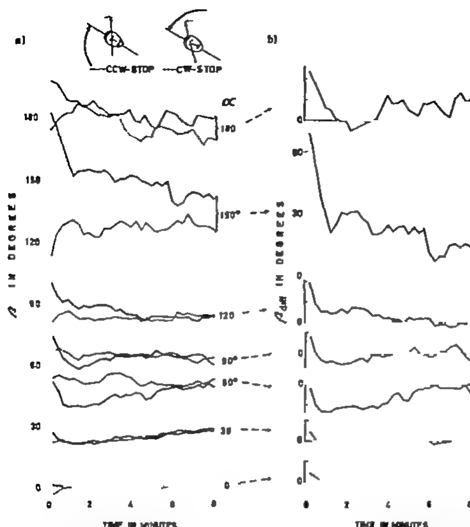


Fig 3 Apparent vertical (angle β) as a function of time at different stop positions averaged for the results of all Ss. (*a*) β as a function of time separately for CW and CCW-experiments (cp. inset figure CW and CCW refers to direction of rotation as seen from S). (*b*) difference of β (β_{diff}) between the corresponding CW and CCW-curves of ( ) as a function of time. The mean of the initial values of β_{diff} at the 0° and 30° positions differs significantly from that at the 150° and 180° positions ($p<0.05$ for each of 5 of the 7 Ss, $p<0.001$ for all 7 Ss combined).

eyes closed for 5 sec. Then a luminous line was adjusted to apparent verticality at 15 sec intervals for 8 min, with the first adjustment 15 sec after stop (t_0) Between the adjustments the line was moved to and fro The starting position of the line and the test order were randomized, with two or three tests daily for each S From the time course of the difference between the adjustments after CW and after CCW rotation (average values of all tests) the effect from the canals was estimated by means of exponential regression analyses.

RESULTS

Nystagmus

All Ss showed a consistent increase of the number of beats and of nystagmus duration with change of stop position from 0 to 180 (Fig 2 *a, b*)

The analysis of the inter-beat times suggested that both the time constant and the initial velocity of slow phase changed with stop position. the greater the degree of tilt at stop, the greater the initial velocity and the slower its decline in time The average time constant

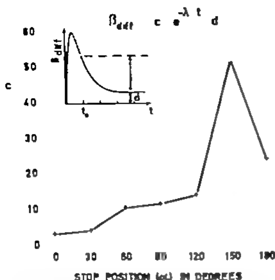


Fig 4 The effect (c) of the canals on the AV at t_0 (i.e. 15 sec after stop) as a function of stop position. Using the formula in the figure, c was calculated by means of exponential regression analyses of the β_{diff} values as presented in Fig. 3 *b* 1/2, time constant explanation of other signs, see inset figure.

of all experiments amounted to about 8 sec. The results of this time analysis, however should be considered with some reserve, because of the limitations of the recording technique.

Apparent vertical

In Fig. 3 *a* the AV is plotted against time separately for the different stop positions and for CW and CCW rotation. As Fig. 3 *b* demonstrates, the difference between the CW and CCW values has clearly increased at the inverted positions (150 180) A more refined picture is acquired from the exponential regression analyses (see formula in Fig. 4) only the values up to 90 sec after stop were used. In order to give a passable fit of the calculated curves an additional constant (*d*) had to be added for each position it represents the CW–CCW difference remaining 1–2 min after stop (cp Fig. 4 inset) As the main diagram of Fig 4 indicates, the initial value of the canal effect (*c*) increased clearly with increasing degree of tilt at stop it reached a maximum at 150

Although for an accurate calculation of the time constant the time intervals of 15 sec are too long, nevertheless useful results could be obtained. The time constant values of the inverted positions were greater than those obtained in the normal range of tilt: for the positions 0–120 the average time constant was 10 sec, for 150 and 180 it was about 30 sec.

A special note should be made about the 150 position. Here for some Ss two distinctly different ways of adjusting were possible one following the same trend as that from smaller degrees of tilt, the other involving adjustments in approximately the symmetry plane of the body (cp also Fischer 1930) The choice between these two was influenced by the direction of the stop impulse stopping after CW rotation resulted more in the one type, stopping after CCW rotation more in the other thus producing the high remaining difference (*d*) at this position (cp Fig. 3 *b*)

DISCUSSION

Nystagmus

The results of the nystagmus experiments resemble those on rabbits of Janeke (1968) who also found an increase in the number of beats and of nystagmus duration from 0 to the 180 position.

Apparent vertical

The canal effect on the AV increased with increasing degree of tilt at stop This confirms the finding of von Holst & Grisebach (1951) of a difference in the effect in the 0 and 180 position. Our results further indicate that the effect is strongest at the 150 position the two adjustment types of the AV may have contributed to the large effect in this position.

General

The *time constant* of nystagmus and AV showed about the same range the values are in agreement with those measured by Melvill Jones *et al* (1964) and Benson & Bodin (1966 *b*) The *duration* of both responses differed much more the case ... of

the AV lasted up to 45 sec (cp Fig. 3 *b*) whereas nystagmus lasted only 5–15 sec (cp Fig. 2 *b*) The canal-induced change of the AV therefore seems to have a lower threshold than nystagmus. An analogue is found in turning chair experiments with stop after yaw rotation in a horizontal plane. the nystagmus lasts shorter and has a higher threshold than the apparent movement of a point of light (the oculogyral illusion" for review see Howard & Templeton, 1966)

The data indicate that with increasing de gree of tilt (1) the suppression of nystagmus decreases, and (2) the canal-induced change of the AV increases. The results fit the hypothesis of the declining effectiveness of the statolith organs (cp introduction)

As to the origin of the declining effective ness we may consider the idea of the "blind spot" of the statolith organs (Quix & Wernd ley 1924 Quix & Eijsvogel, 1929) These au thors assumed that the pressure of the statoconial membranes is the effective stimulus for the receptors. The "blind spot" concerns the stimulus situation in those inverted positions in no macula exerts pressure and therefore statolith organs would be out of action. -r as it is now generally accepted (cp. Trincker 1962) that the macular receptors are not stimulated by pressure but by shear force we must drop the "blind spot" idea.

Beyond that, any peripheral explanation has to be doubted. Recent measurements of Trinc ker (pers. comm.) indicate that the discharge rate of single utricular afferents is almost as regular in the 180 as in the 0 position. Consequently the effectiveness of the statolith or gans is not likely to depend on the stability of the peripheral afference Further research is therefore needed to ascertain which physiological processes underly the decline in the ef fectiveness of the statolith organs.

It might be worth noting that the "break down" of the effectiveness occurs in positions taken up only occasionally by humans, in which therefore accurate functioning of the space orientation system is not required.

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ZUSAMMENFASSUNG

Die Wirkung von postrotatorischer Reizung der (er tikalen) Bogengänge auf Nystagmus und subjektive Vertikale wurden als Funktion der Stoplage (Körper neigung) untersucht, die die Versuchsperson in einer vertikal ausgerichteten Frontal-Ebene ihres Kopfes einnahm. Sowohl Dauer und Zahl der Schläge des Nystagmus, als auch die Wirkung auf die subjektive Vertikale wuchsen mit Zunahme der Körperneigung sie erreichten ein Maximum in den Kopf-unten-Lagen. Die Ergebnisse werden im Sinne einer abnehmenden Effektivität der Statolithenorgane mit Zunahme der Körperneigung gedeutet und in Zusammenhang mit ähnlichen Befunden diskutiert.

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ULTRASTRUCTURAL LOCALIZATION OF DPNH-DIAPHORASE IN THE COCHLEA

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Submicroscopic localization of DPNH-diaphorase (*NADH-cytochrome C reductase*) *in the glutaraldehyde* prefixed inner ear of the laboratory animals was studied utilizing TNBT (tetranitro blue tetrazolium) as an indicator The strongest reaction was found in the mitochondria of the outer hair cells. The enzyme reaction was also found on the endoplasmic reticulum of the outer and inner hair cells, epithelial cells of the stria vascularis, Reissner's membrane, and connective tissue cells of the spiral ligament and limbus. Afferent nerve endings demonstrated diffuse enzyme reaction in their cytoplasm, however efferent nerve endings showed dense reaction along the double membrane located opposite the nerve ending inside of the hair cells. Some of the interdental cells showed reaction in their cytoplasmic contents and showed differences of intensity of reaction among the interdental cells, possibly indicating different stages of enzyme activity

The distribution of oxidative enzymes in the sensory cells of the inner ear should be of considerable significance in understanding the biosynthesis of chemicals incorporated in the process of hearing. It is assumed that high energy is needed to transduce the physical stimuli into chemical energy at the terminal sense organ, namely the hair cells of the organ of Corti.

Many investigators, mostly using light microscopy attempted to demonstrate, histochemically and cytochemically the activity of oxidative enzymes in the inner ear (Vosteen, 1960· Gerhardt, 1961 Spoendlin & Balogh 1963 Vinnikov & Titova, 1964 Koide *et al.*, 1964 Nomura & Balogh, 1964 Nakai, 1965 and others) Recently Nakai & Hilding (1968), utilizing TNBT (tetranitro blue tetrazolium) as an indicator demonstrated succinic dehydrogenase and DPNH-diaphorase localization in the unfixed inner ear at the electron microscopic level.

The investigation reported here was carried out as a parallel study of the enzyme changes in ears under conditions of auditory fatigue. Its purpose is to demonstrate the distribution of DPNH-diaphorase in the prefixed cochlea of the normal guinea pig and chinchilla on the light and electron microscopic levels utilizing TNBT as an electron acceptor

MATERIAL AND METHOD

Thirty temporal bones of ten normal guinea pigs and five chinchillas were subjected to a light and electron microscopic examination after incubation for enzyme reaction As soon as the temporal bones were removed from the animal, they were prefixed in refrigerated 2% glutaraldehyde in 0 1 *M* phosphate buffer solution at pH 7 4 for one hour The bullae of the temporal bones and the apical portion of the cochlea were opened to allow the fixative to flow into the membranous labyrinth. At the same time the stapes was removed and a fine pipette was used to agitate the fixative into

DPNH (diphosphopyridine nucleotide, reduced form)· same as NADH-cytochrome C reductase (nicotinamide-adenine dinucleotide, reduced form).

the cochlea and vestibule to obtain maximum preservation of the membranous structure. Following the prefixation, the temporal bones were rinsed in cold 0.3 *M* sucrose in 0.1 *M* phosphate buffer at pH 7.4. After 4 changes of the buffer solution over a period of one hour the temporal bones were incubated in the substrate solution which included the indicator TNBT in DMF (dimethyl formamide) for 1 hour at a temperature of 37 C. To make the substrate solution, this TNBT in DMF solution was mixed with an equal part of DPNH in phosphate buffer solution. The solution was filtered before it was used. During this incubation the temporal bones were agitated gently with fine pipettes. A control temporal bone was incubated in a solution, which did not contain reduced diphosphopyridine nucleotide (DPNH) to exclude the possibility of misinterpretating a nonspecific reduction of TNBT. After the incubation the temporal bones were washed in 0.3 *M* sucrose solution and then the specimen was fixed in 1% osmic acid for one hour. After the fixation in osmic acid the temporal bones were dehydrated in graded alcohol, embedded in epoxy resin and hardened in the oven.

Following the hardening the temporal bones were cut in half through the axis of the midmodiolus by a jewler's saw. Each turn of the cochlea was separated and remounted on an empty epon capsule. After trimming, each turn of the cochlea was sectioned between 1 and 3 microns thick and mounted on a glass slide for phase contrast light microscopic examination. The tissues were not stained therefore, any colored pigment could easily be recognized. Representative areas were selected for further thin sectioning for electron microscopic examination. This sectioning was carried out by an LKB ultratome. The thickness of the thin-sectioned specimens varied from 60 to 90 Angströms. Thin sections were then stained only with alcoholic uranyl acetate. These sections were picken up on copper grids coated with a formvar membrane. The electron microscopic investigation was done on the RCA EMU 3 F electron microscope with magnification varying between ×1000 to 30,000.

RESULTS

Light microscopic findings

The intensity of DPNH-diaphorase distribution in the cochlea was found in the following order (from most to least) the outer hair cells, inner hair cells, Reissner's membrane, superior and inferior aspects of spiral ligament, mesothelial-like cells of the basilar membrane, connective tissue cells of the spiral ligament, interdental cells, connective tissue cells in the spiral limbus, nerve fibers and endings and infrequently in the epithelial cells of stria vascularis (Figs. 1 and 6).

These findings were rather consistent in all turns. With the light microscope the distribution of TNBT formazan seems to coincide with the distribution of the mitochondria in the outer and inner hair cells. However in Reissner's membrane, the interdental cells, the spiral ligament and the basilar membrane, the enzyme distribution seems to be somewhat diffuse. Occasionally the enzyme reaction was found in the pillar of the pillar cells. It is also interesting to observe that some interdental cells in one microscopic field seemed to show strong enzyme activities, whereas, others did not show positive enzyme stain (Fig. 9).

Mesothelial-like cells covering the scala vestibuli just above the Reissner's membrane attachment also showed strong reaction (Fig. 6 A and B). There were no noticeable differences of enzyme reaction between chinchilla and guinea pig cochleae.

Electron microscopic findings

The strongest reaction was noted in the mitochondria of the outer hair cells and to a lesser extent along the endoplasmic reticulum. The appearance of a reaction resembled either a fine linear aggregation or a diffuse dust-like aggregation (Figs. 2, 3 and 4). Quite frequently the reaction was seen along the microsomes resembling exaggerated ribosomes. The

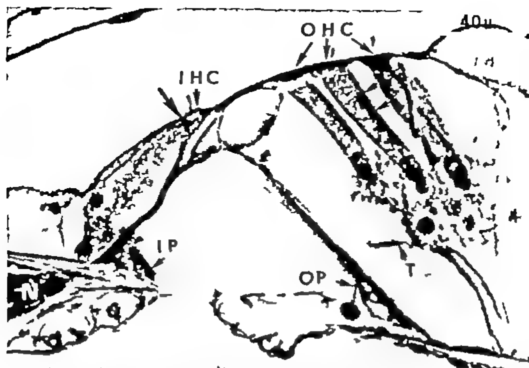


Fig 1 A phase contrast micrograph of the organ of Corti of a chinchilla illustrating DPNH-diaphorase distribution in the outer and inner hair cells. The reaction is stronger at the base and along the wall of the outer hair cells (arrows), whereas, in the inner hair cell a weaker reaction can be observed in the upper part of the cell (arrow). Occasionally the nerve ending region of the outer hair cell, the unmyelinated nerve fibers under the outer and inner hair cells, the tunnel fibers and myelinated nerve fibers were stained. *I* inner hair cell, *O* outer hair cells, *T* radial tunnel fiber *IP* inner pillar cell *OP* outer pillar cell *N* myelinated nerve fiber

inner hair cell mitochondria also showed diaphorase reaction but it was much weaker than that of the outer hair cells

The sub-synaptic cisterna of the outer hair cell frequently showed diaphorase reaction along its membranous structure (Fig. 3) Whether the enzyme reaction in the nerve ending was present or not remained controversial on the light microscopic level. Electron microscopically there were indications that some of the nerve endings did react. There was no clear cut mitochondrial localization in these nerve endings, rather the reaction was found diffusely in the cytoplasm (Fig. 4)

Occasionally myelinated nerve fibers showed moderate enzyme reaction The reaction deposits seemed in some instances to be related to the mitochondria (Fig. 5) The big lipid granules of the Hensen's cell showed a strong reaction along its surface. However this reaction of the myelin sheath and Hensen's cells can be observed among the control group which does not contain a substrate suggesting these reactions could be nonspecific.

Reissner's membrane showed strong reaction with a distinct DPNH-diaphorase distribution pattern found more closely associated with the endoplasmic reticulum than with the mitochondria (Fig. 7)

A few epithelial cells of the stria vascularis stained positively while the majority did not. When there was a reaction it was confined to the endoplasmic reticulum (Fig 8 A)

The interdental cells showed inconsistent reaction Some interdental cells showed very strong reaction while the remaining did not stain (Fig. 9 A) The electron microscopic examination revealed strong diaphorase distribution along the endoplasmic reticulum and microsomes (Fig. 9 B) A mitochondrial distri-

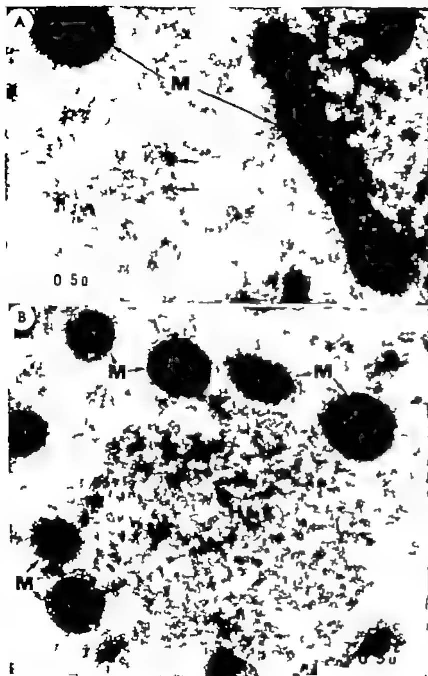


Fig. 2 (A) Mitochondria of the inner hair cell of the guinea pig shows intramitochondrial formazan deposits. Arrows indicate formazan deposits in the cytoplasm possibly related to microsomes. *M* Mitochondria.

(B) An electron photomicrograph of the outer hair cell (guinea pig) showing Hensen's body (*H*). Numerous mitochondria are *making contact* with the Hensen's body. Observe dirt-like formazan particles which indicate *DPNH*-diaphorase in the mitochondria. *M* Mitochondria.

bution can be discerned among the interdental cells but it is less intense than that found in the endoplasmic reticulum.

The connective tissue cells in the limbus spiralis showed a strong reaction. The distribution in these cells seemed to be related closely with the endoplasmic reticulum and to a lesser degree with the mitochondria (Fig. 10). When the endoplasmic reticulum is engorged, the enzyme precipitates are seen on the swollen membrane (Fig. 10 A). These connective tissue cells supposedly secrete fibers of the limbus.



Fig 3 The base of outer hair cell with efferent nerve ending (*NE*). The formazan particles are condensed along the synaptic bar (arrows). The diaphorase can be observed in the mitochondria (*M*) of the outer hair cell (*OHC*).

DISCUSSION

Dehydrogenases participate in many steps of the Kreb s tricarboxylic cycle. The metabolism of carbohydrates, fats and proteins cannot be completed without these enzymes. The methods for demonstrating diaphorase and DPN or TPN-dependent dehydrogenase are related in principle since the demonstration of a specific dehydrogenase is accomplished through the diaphorases (Barker & Anderson, 1963) Because of the diaphorase activity of cytochrome C reductase preparations, and of their similar physical properties and distribution, it has been suggested that DPNH-cytochrome C reductase and DPNH-diaphorase are related enzymes. The exact nature of this relation still has not been completely resolved (Garfinkel, 1957 Massey 1958) For the foregoing reasons diaphorase activity in the inner ear bears considerable significance in the understanding of cell respiration which provides energy for cellular activity

Since histochemistry and cytochemistry can be technically intricate, one may raise questions regarding the methodology used in this investigation and its interpretation. With these questions in mind, I would like to discuss some of the rationale of the methods employed in this study Various investigators (Sedar & Rosa, 1959 Sedar *et al.*, 1962 Barrnett, 1959 Scarpelli, 1961) have adopted "tetrazolium salt" to demonstrate succinic dehydrogenase and DPNH or TPNH-diaphorase distribution. The tetrazolium salt which is colorless will turn into blue formazan particles upon the acceptance of electrons released in the process of dehydrogenation. These particles are insoluble and can be clearly resolved with both the light and electron microscopes.

The formazan particles resulting from conventionally used nitro blue tetrazolium (NBT) vary in size between 300 to 1000 A. The large size of the deposits precluded intramembranous localization of dehydrogenase enzyme systems in the mitochondrial cristae. Studies by Novikoff *et al.* (1961) and Nachlas *et al.* (1957) have pointed out certain difficulties in using NBT for staining intracellular structures which are in close proximity to lipid aqueous interface, and the tendency for this particular dinitro-formazan (DNF) to crystallize in histochemical preparations has been recognized for some time. More recently with TNBT as the electron acceptor it has been possible by virtue of the smaller formazan deposit formed, to resolve succinic dehydrogenase enzyme active sites measuring 30 to 40 A in the osmiophilic lamellae of cristae of mitochondria (Sedar *et al.*, 1962) Rosa & Tsou (1963) compared these



Fig 4 The afferent nerve endings (*NE*) of the outer hair cell (*OHC*) showing diffuse distribution of formazan particles in its cytoplasm.

two tetrazoliums, NBT and TNBT in regards to their cytochemical behavior and concluded that TNBT is, in spite of certain peculiar features, a more suitable salt than NBT

Although acceptable localization of cytochemical reaction such as succinic dehydrogenase (SDH) in heart muscle (Barrnett & Palade, 1957) and SDH and NADH-cytochrome C reductase in rat kidney (Scarpelli, 1961) were reported, there was some criticism concerning the use of fresh tissue for the cytochemistry (Scarpelli & Kanczak, 1965)

A systematic investigation of a variety on mono- and dialdehydes as pote tial f atives for ultrastructural cytochemistry by Sabatini and his associates (1963) has led to the introduction of several additional substances which are capable of preserving both fine structure and enzyme activity The aldehydes which include glutaraldehyde are apparently active enough to preserve both fine structure and enzyme activity (Seligsberger & Sadler 1957 Fein & Filachione, 1957) Scarpelli & Kanczak (1965) have successfully demonstrated such enzymes as cytochrome oxidase and myofibrillar ATPase using glutaraldehyde prefixed tissue Our results, using a glutaraldehyde pre xed cochlea for incubation to demonstrate



Fig 5 An electron photomicrograph of the myelinated nerv fiber near the habenula perforata. One mitochondria (*M*) shows aggregation of the formazan particles in the cristae of the mitochondria.

DPNH-diaphorase reaction, was in agreement with Scarpelli & Kanczak (1965) in terms of fine morphology and an appreciable enzyme reaction.

In view of the findings that TNBT permitted fine localization of the enzyme activity and that the glutaraldehyde prefixation provided superior preservation of tissue, the technique employed in this study was felt to be justified.

Our finding is in agreement with Nakai & Hilding's (1968) observation that the formazan deposits are found in the mitochondria and along the cisternal lamellar structure of the outer hair cell wall which is believed to be a modified endoplasmic reticulum. The positive enzyme reaction along the cisternal lamellae is supported by the biochemical evidence that endoplasmic reticulum contains DPNH-cytochrome C reductase (Ernster *et al* 1962) This enzyme system is also capable of reducing tetrazolium (Novikoff 1963) Quite frequently an intense diaphorase reaction could be observed at the Hensen's body of the outer hair cells. Hensen's body is also interpreted as the extension of the endoplasmic reticulum and often surrounds the mitochondria. Bourne (1962) speculated that when the mitochondria are completely surrounded by the endoplasmic reticulum, it is a result of glucose passing through the endoplasmic reticulum membrane, undergoing glycolysis there, and the glycolytic product feeding directly to the mitochondria.

Nomura & Balogh (1964) compared the distribution of DPNH and TPNH-diaphorase in the cochlea prepared by frozen sections and those prepared by perfusion utilizing NBT as the electron acceptor There was a decrease of enzyme activity among the perfused cochleae The decrease of enzyme activity was particularly evident in the stria vascularis. This brings up the serious question of whether we are losing any free (non-structure bound) or microsomal enzymes. This enzyme loss might be attributed to penetration of the stria vascularis, or it may be that the free enzymes were washed out during the perfusion and subsequent tissue preparation.

Bourne & Allen (1941) postulated that most of the process of respiration and glycolysis actually takes place in the cytoplasm. They noted that differential centrifugation of cell homogenates has demonstrated that most of the enzymes responsible for the glycolytic cycle have been found to be present either in the supernatent or in "microsomes" and those concerned with the cytochemical system and Kreb's cycle are localized specifically in the

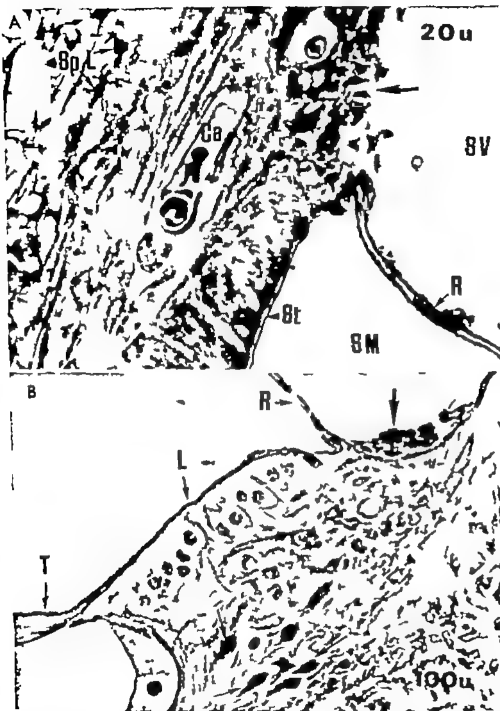


Fig. 6. (A) A phase contrast micrograph of the guinea pig showing the upper part of stria vascularis where the Reissner's membrane attaches. A strong diaphorase reaction can be observed on the mesothelial-like cells on the side of scala vestibuli (arrow). Some reaction can also be observed in the spiral ligament, the epithelium of the stria vascularis and Reissner's membrane. *SV*, Scala vestibuli; *R*, Reissner's membrane; *SM*, scala media; *St*, stria vascularis; *SP.L.*, spiral ligament; *Ca*, capillary.

(B) Epithelial cells covering the scala vestibuli above Reissner's membrane attachment near the limbus show strong enzyme reaction (dark arrow). Small arrows are pointed to the fibrocytes of the limbus, which also show enzyme reaction. *R*, Reissner's membrane; *L*, limbus; *T*, tectorial membrane.

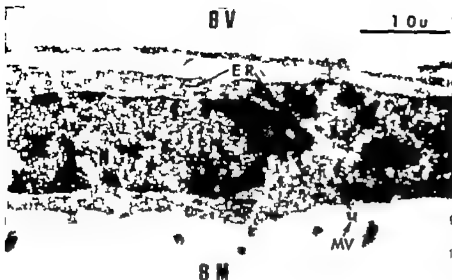


Fig 7 An electron micrograph of Reissner's membrane of the guinea pig showing diaphorase distribution along the endoplasmic reticulum (*ER*). *MV* Microvilli *N* nucleus *SV* scala vestibuli *SM* scala media.

mitochondria. In view of these findings, the commonly held view that discrete histochemical localization limited to small cytoplasmic foci are necessarily more accurate than diffuse ones, must be abandoned. As pointed out by Nachlas *et al* (1957) widely-dispersed cytoplasmic deposits of reaction products may indeed represent a true distribution of an intra- substrate However it should be added thus only applies when it is assured that ner the enzyme nor the reaction product iffused from a focus of concentration and that the histochemical reagent or reaction product had not been preferentially bound to certain intracellular sites Unfortunately the absence of these conditions is difficult to establish. Nevertheless, the view that the diaphorase distribution is diffuse in the outer hair cells and stria vascularis in frozen sections is not without support. Since microsomes and endoplasmic reticulum are abundantly found in outer hair cells, it also could be postulated that the diffusion of diaphorase in the outer hair cells might be the result of the diffuse distribution of these cytoplasmic structures

The enzyme reaction present in the nerve ending and nerve fiber is controversial Spoendlin & Balogh (1963) reported that there is no formazan deposit in the nerve endings in the organ of Corti when incubated for succinic dehydrogenase On the other hand, Vosteen (1960) showed very strong succinic dehydrogenase reaction in the nerve endings. Gerhardt (1961) reported that there is a diffuse scattering of formazan particles in the cytoplasm of the nerve endings and also of the unmyelinated portion of the nerve fibers Our material suggests the reaction in the nerve endings is much less intense than the outer hair cell body with some evidence of diffuse distribution in the cytoplasm.

Strong activities of succinic dehydrogenase (Spoendlin & Balogh, 1963 Koide *et al*, 1964) and diaphorase (Nomura & Balogh, 1964) in the upper and lower portion of the spiral ligament has been reported, while the mid-portion was considerably weaker This finding could be attributed to the fast penetration rate of the incubation media from the scala vestibuli, where the incubation media were introduced The other possible explanation can be made by the fact that they are enzyme active. The external sulcus behind the spiral prominence always contains abundant vascularity and therefore, it is concervable that this area could participate in an active metabolic role.

The enzyme reaction in the interdental cell



Fig 8 (A) An electron photomicrograph of the stria vascularis illustrating diaphorase distribution along the endoplasmic reticulum (arrow). *M* Mitochondria.

(B) An electron micrograph of normal stria vascularis showing abundant mitochondria in the epithelial and intermediary cells of the stria vascularis. *M* Mitochondria.

is noteworthy It is quite often observed that some interdental cells are stained while the remaining are free from the staining. It might indicate that all the interdental cells are not in the same stage of metabolism. This fact is also supported by the morphological findings of these cells (Lim & Lane, 1969) It is conceivable that the level of enzyme activity of one cell might be different from that of another at any given time The level might be geared to the rate limiting factor of glycolysis

The overall result of this study suggests that there may be possible differences of enzyme foci among the organelles of different cochlear cells. Same results have been observed in the cells of other organs In glutaraldehyde-fixed liver and kidney the formazan deposits were largely limited to the membranes of the endoplasmic reticulum and the external membrane of mitochondria, whereas, in myocardium, deposits were found in endoplasmic reticulum and within the mitochondria in relation to the

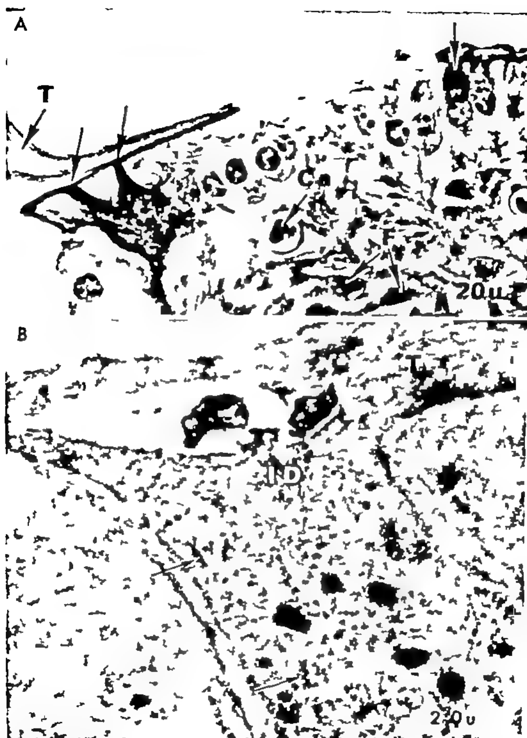


Fig 8. (A) A phase contrast photomicrograph of the limbus spiralis of the guinea pig. Strong diaphorase reaction can be observed in certain interdental cells (arrow), whereas, remaining interdental cells are free from reaction. Also, some connective tissue cells of the limbus (*F*) are strongly reacted. *T* Tectorial membrane; *Ca*, Capillary

(B) The upper part of the interdental cell (*ID*) is shown. The formazan particles seem to be distributed along the endoplasmic reticulum (arrows) and microsomes.



Fig 10 (A) An electron micrograph of the connective tissue cell of the limbus (guinea pig) showing engorged endoplasmic reticulum (arrows). Extensive diaphorase concentration can be observed on the surface of endoplasmic reticulum. *N* Nucleus.

(B) An electron micrograph of the connective tissue cell of the limbus showing extensive diaphorase reaction along the endoplasmic reticulum and diffusely in the cytoplasm. Some mitochondria show weak reaction. *ER* Endoplasmic reticulum *M* Mitochondria.

cristae. Mitochondrial preference was distinct in the hair cells of this material. But in the interdental cells, connective tissue cells of the spiral ligament and of the limbus spiralis, the epithelial cells of the stria vascularis, and Reissner's membrane there seems to be more related to the endoplasmic reticulum than to the mitochondria.

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ZUSAMMENFASSUNG

e submikroskopische Lokalisierung von DPNH iaphorase (NADH-Cytochrom C Reduktase) wurde mittels Vorfixierung mit Glutaraldehyd und Anfärbung mit TNBT (Tetranitroblau Tetrazolium) als Indikator am inneren Ohr von Versuchstieren untersucht. Am stärksten trat die Reaktion in den Mitochondrien der äusseren Hörzellen hervor. Das Enzym war auch im Endoplasma der äusseren und inneren Hörzellen, Epithelialzellen der Stria Vascularis, Reissners Membran, und in den Bindegewebezellen der Ligamentum Spirale Cochleae und Limbus Spiralis nachweisbar. In zuführenden Nervenden ist die Enzymaktivität diffus durch das Protoplasma verstreut, während in abführenden Nervenden die Reaktion in der doppelten Membran, die [illegible] den Hörzellen gegenüber den Nervenden liegt, konzentriert war. Manche der Interdentalzellen gaben auch eine Reaktion im Protoplasma, die von unterschiedlicher Intensität war und durch verschiedene Stadien der Enzymaktivität hervorgerufen sein konnte.

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THE ULTRASTRUCTURE OF THE SPIRAL LIGAMENT IN THE RHESUS MONKEY

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Electron microscopy of the spiral ligament of the Rhesus monkey reveals two types of fibrocytes. Type I contains a relatively small number of cell organelles and is found in the deeper part of the spiral ligament and adjacent to the stria vascularis. Type II shows many cell organelles and is located near the surfaces of both scalae vestibuli and tympani, behind the spiral prominence epithelium and external sulcus cells. Fibrocytes establish numerous attachments (fascia occludens, fascia adherens, and macula adherens) with each other and even between the cell processes of the same cell. Type II fibrocytes appear to be actively engaged in fluid metabolism, and often ntain long mitochondria with longitudinally ar d cristae. Connective tissue channels are ob- ved between the external sulcus cells extending to the cells of Claudius. The physiological importance the external sulcus cells is considered and discussed.

The first ultrastructural study of the connective tissues in the cochlea is accomplished in the rat by Iurato (1962) A similar study of the perilymphatic tissue of the vestibule of the rat is reported by Hamilton (1967) Our study of the spiral ligament from the cochlea of the Rhesus monkey indicates some morphological differences from those reported earlier The present study concentrates on four areas of the spiral ligament of the Rhesus monkey and intends to provide useful information on its function and also to establish a basis for effective evaluation of human material.

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MATERIALS AND METHODS

Four adult Rhesus monkeys (average weight 5 kg) and one fetus were used in this study Animals were anesthetized with an intraperitoneal administration of pentobarbital sodium (25 mg/kg) The middle ear was surgically exposed and, after removing the footplate, 1% phosphate buffered osmium (pH 7.3) was injected and agitated through the oval window Animals were terminated immediately after injection of the fixative The cochlear apex of the excised ear was opened and the fixative again agitated through the cochlear apex, oval window and punctured round window membrane

The cochleae were embedded in Epon, and trimmed specimen sections were cut on a plane parallel to the long axis of the modiolus with an LKB ultrotome The entire area of the spiral ligament, except the area adjacent to the endosteal layer was examined and photographed with a Siemens Elmiskop 1 ranging from 1500 to 36,000 × magnification.

FINDINGS

Spiral ligament adjacent to the scala vestibuli

The spiral ligament in this area is composed of mesenchymal epithelial cells, fibrocytes, capillaries, and intercellular substances. The

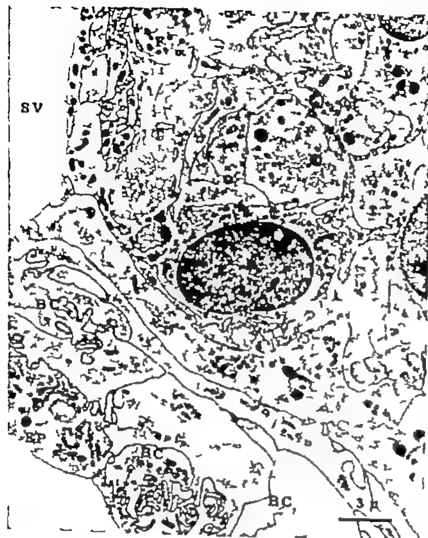


Fig. 1. The spiral ligament at the Reissner's membrane attachment area. The fibrocytes (*F*), Type II are tightly organized at the scala vestibuli (*SV*) as well as in the deep part. The epithelial cell (*EP*) of the Reissner's membrane when underlined by basal cells (*BC*) loses the basal lamina. (*T*) Transitional epithelium. 4340

mesenchymal epithelial cells, the same type of cell observed in Reissner's membrane, are few in number and are scattered. Most of the surface of the scala vestibuli is covered with fibrocytes and their parallel layers of cell processes. The cell layers are more compact near Reissner's membrane (Fig. 1) but reduced in number and showing wider intercellular spaces (Fig. 2) in the upper distal portion. The fibrocytes demonstrate numerous attachment areas (Fig. 3): fascia occludens which shows fusion of the outer leaflets of the unit membrane, occasionally macula adherens which demonstrates a thickening of the plasma membrane and fascia adherens where adjacent cytoplasmic condensation is noted. The basal lamina is not found below these cell layers.

In both superficial and deep parts, the fibrocytes show variations in cytological details. The fibrocytes near the fluid surface tend to show more organelles than others: they contain many rod-shaped mitochondria (some of which show longitudinal cristae), numerous vesicles, Golgi apparatus, lysosomes, and some filaments. The cell organelles are particularly rich around the nucleus. There are many long and irregular cell processes: one process may show many cell organelles while other processes of the same cell may show few.

One of the interesting aspects of fibrocytes



Fig 2 The spiral ligament above the Reissner's membrane attachment. Surface area shows discontinuity of cell layers and a capillary includes one to two layers of pericytes. Note fibrocytes (F), Type II, contain numerous mitochondria and vesicles. 4340. The *inset* shows a higher magnification of fibrils (100 to 120 A diameter) found on the surface of the fibrocyte in the scala vestibuli. Corresponding fibrils are indicated by arrows. 39,000

located near the scala vestibuli is that bundles of fibrils are found close to the plasma membrane facing the lumen (Fig. 2) Such fibrils are identical to those seen in the deeper part. Below the surface, the fibrils are scattered and crisscrossed (Fig. 4 A) in both diffuse and bundle forms. When transversely cut, each fibril (100 to 120 A) appears in rectangular form (Fig. 4 B) 130 to 230 A apart. The fibril is surrounded by a light zone and is imbedded in a diffuse electron dense substance, and often seems to be composed of four or more subunit filaments. Some fibrils appear larger and round in shape. Fibrils are not seen in direct contact with the plasma membrane of fibrocytes however when the bundles of fibrils are found near the membrane, there is occasionally a cytoplasmic condensation. Bundles of fibrils are found frequently within deep invaginations formed in the cell body and/or surrounded by protruding cell processes The plasma membranes adjacent to the fibrils show invaginations forming pockets which contain a diffuse substance similar to the interfibrillar matrix (Fig. 4 B) When two cell processes of the same cell overlap infolding fibrils, fascia occludens and macula adherens are established between them (Fig. 4 B). Collagen fibers of band form are not observed.

Capillaries near the attachment of the Reissner's membrane are partly surrounded by pericytes, fibrocytes and fibrils. The endothelial cells show no fenestration, and lack a smooth muscle layer as well as neural elements. The

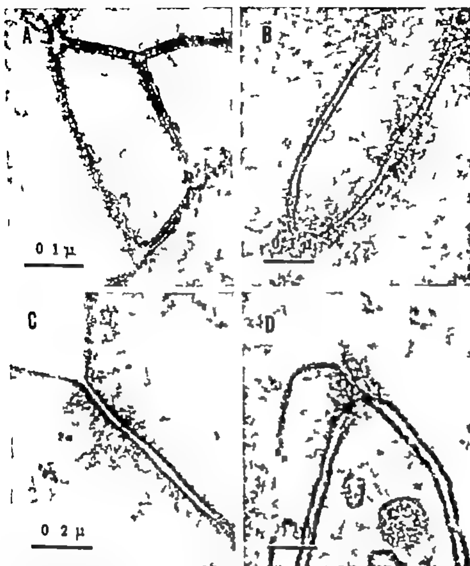


Fig. 3 (A). The most common type of fibrocyt junction, fascia occludens. Note differences between this junction and that of mesenchymal epithelial cells (*D*). 159,000.

(B) The second type of junction seen between fibrocytes. Note cytoplasmic condensations adjacent to dilated space and fascia occludens on both sides of this macula adherens. 122,000.

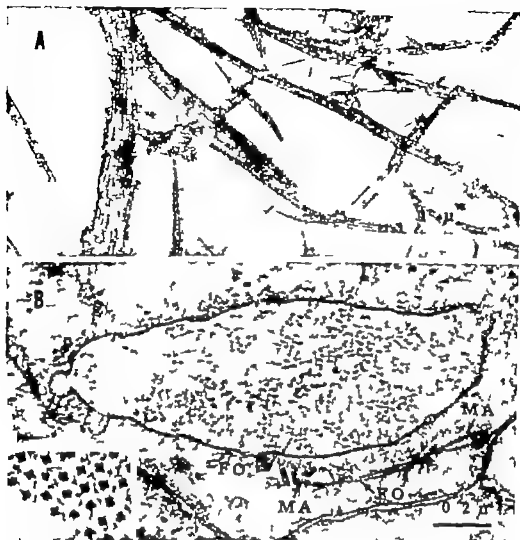
(C) The third type of fibrocyte junction, fascia adherens, with cytoplasmic condensation without intermediate line and dilated space. 82,000

(D) Mesenchymal epithelial cells form macula adherens at the cell corners. Note the cytoplasmic condensations and an intermediate line. 126,000.

vessel wall is indirectly exposed to perilymph of the scala vestibuli through irregular inter cellular spaces (Fig. 2)

Spiral ligament adjacent to the stria vascularis

The basal cell layers establish a demarkation between the stria vascularis and the spiral ligament (Fig. 5 A) The basal cell layers are a few cells thick and lie parallel to the luminal surface of the stria vascularis. They abut the basal part of the endolymphatic epithelium of Reissner's membrane on one side and the spiral prominence epithelium on the other side. The entire area covered by the basal cells lacks



g 4 Intercellular fibrils of the spiral ligament. (A) A typical arrangement of fibrils found in the deeper part. They crisscross in a wide fluid space 32,000.
(B) Two cell processes of the same fibrocyte surround fibrils and establish the fascia occludens (*FO*) and macula adherens (*MA*). Note the rectangular shape of each fibril surrounded by a light zone (*insert*) and pits (*P*) containing homogeneous substance. 70,000.

the basal lamina this is the only area in the scala media lacking a basal lamina beneath the epithelial lining, except the habenula perforata. However a short basal lamina-like substance is sometimes found between the basal cells and fibrocytes, and even behind the fibrocytes. Outstanding features of the basal cells are that they contain numerous filaments, are rich in RNA particles and large osmiophilic bodies, and establish numerous cell junctions, macula adherens and fascia occludens, between them. Cell junctions are relatively sparse between the marginal cells of the stria vascularis and the basal cells.

Below the basal cells are one to three layers of fibrocytes in an arrangement similar to the basal cells and almost equal or slightly greater in thickness. In comparison to the basal cells, these fibrocytes are light in cytoplasmic density though sometimes differentiation is obscure particularly in the first row adjacent to the basal cells. The cell membranes between

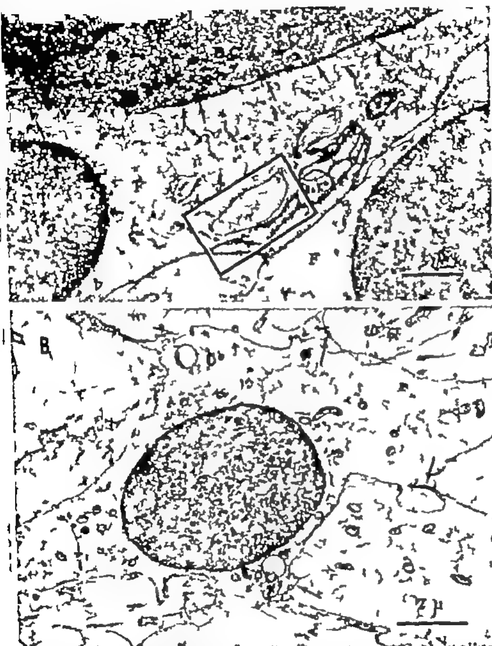


Fig. 5. (A) The spiral ligament behind the stria vascularis. The basal cells (*BC*) appear dense containing numerous filaments in comparison to fibrocytes (*F*). The basal cells are tightly arranged while the fibrocytes are loose. Many macula adherens (*arrows*) are seen between the basal cell and the fibrocyte. 15,000. A higher magnification of the area in brackets is shown in Fig. 4 B.
(B) Typical fibrocyte (Type I) located in the deep part of the spiral ligament containing few cell organelles. Note cell junctions (*arrows*). 9000

the basal cell and fibrocyte run parallel, demonstrating numerous attachment areas. The space between them is very narrow; however at both ends of the stria vascularis are wide spaces in which bundles of fibrils and a diffuse electron dense substance are found. There are two types of cell junctions between basal cells and fibrocytes: the *fascia occludens* (Fig.



Fig 6 The spiral ligament behind the spiral prominence epithelium.

(A) The root portions (*R*) of the external sulcus cells abut the spiral prominence epithelium (*SP*). Note absence of the basal lamina between them (*arrow*). 7500.

(B) Underneath the epithelium are cells (Type II fibrocytes) tightly packed and rich in mitochondria, Golgi network and vesicles. Note the parallel array of long mitochondria with cristae longitudinally arranged. 5000.

3 A) and the macula adherens (Fig 3 B) At the macula adherens the intercellular space is enlarged and oval in shape, and the outer leaflets are separated and between them is an electron dense substance on both sides of the macula adherens the outer leaflets join forming the fascia occludens.

In the Reissner's membrane attachment

area, cellular organization is more complex and compact. Facing the scala media, the epithelial cells of the Reissner's membrane become continuous with the marginal cells, however, another type of cell sometimes adjoins the marginal cells of the stria vascularis. In this transitional area, the epithelial cells are flat, contain small numbers of mitochondria and RNA granules, and show some cytoplasmic infoldings. The basal parts of these cells are closely underlined by a few layers of basal cells (Fig. 1) which are continuous from the stria vascularis and lack the basal lamina. The epithelial cells of this transitional area are called "spindle-formed cells" by Katagiri *et al* (1968). The tight junction is observed throughout the luminal surface from Reissner's membrane to the marginal cells. Fibrocytes in the Reissner's membrane area are rich in vesicles and mitochondria which sometimes show longitudinally arranged cristae. The basal lamina of the Reissner's membrane often ends at the basal cells or extends into the masses of fibrocytes, basal cells and fibrils. It takes a short, wavy course and abruptly terminates.

The fibrocytes deeper in the spiral ligament (Fig. 5 B) are loosely organized and form a complex network with their cell processes extended in all directions. The cell body as well as the cell processes form fascia occludens (Fig. 3 A), macula adherens (Fig. 3 B) and fascia adherens (Fig. 3 C). The fibrocytes contain few mitochondria, vesicles, Golgi network, lipofuscin granules and filaments. The intracellular filaments are many and some appear to run in the same continuous direction as the intercellular fibrils; the continuity of these intra- and intercellular fibrils is not established. The intracellular filaments are smaller in diameter than the intercellular fibrils.

Spiral ligament adjacent to the spiral prominence and basilar membrane

The spiral prominence epithelium is a rather thin cell layer with some lateral plasma membrane infoldings. It is usually underlined with the basal lamina which is continuous from the basilar membrane (Fig. 6 A). The epithelium is partly adjoined below by basal cells from the stria vascularis. In this case, the basal lamina is often fragmentary and is located below the basal cell layer.

Behind this epithelial lining and the basal cells are abundant fibrils and fibrocytes. The fibrocytes show numerous cell processes with some interdigitation and lie adjacent to capillaries and the root portions of the external sulcus cells. They contain many rod-shaped mitochondria, a large concentric arrangement of smooth endoplasmic reticulum, short smooth and rough endoplasmic reticula, and numerous vesicles (Figs. 6 B and 7). Some mitochondria are very large, often lie in parallel array and show longitudinally aligned cristae. Occasionally these huge mitochondria contain a diffuse electron dense matrix and show extensive pleomorphism (Fig. 7) which appears to be a result of their fusion.

The surfaces of the external sulcus cells are often exposed to endolymph (Fig. 8 A), but are partially covered by the spiral prominence epithelium and/or by cells of Claudius. Their luminal surfaces under the cells of Claudius are not continuous; sometimes the basal lamina which is continuous from the basilar membrane loops between the external sulcus cells to lie adjacent to the narrowly exposed part of the cells of Claudius. Thus the "channels" formed by the basal lamina of the external sulcus cells extend to the cells of Claudius at one end and open into the connective tissue space on the other (Fig. 8 B). The "channels" often contain fibrils and the processes of fibrocytes. The external sulcus cells are arranged in clusters and their nuclei are found both near the fluid surface and in deeper zones, including an area behind the spiral prominence epithelium. Their shape is irregular with their cytoplasmic processes divided into a few rootlike extensions toward the zone behind the spiral prominence epithelium even abutting it (Fig. 6 A) and extending behind the stria vascularis. Root processes from different cells converge and penetrate the fibrillar extension

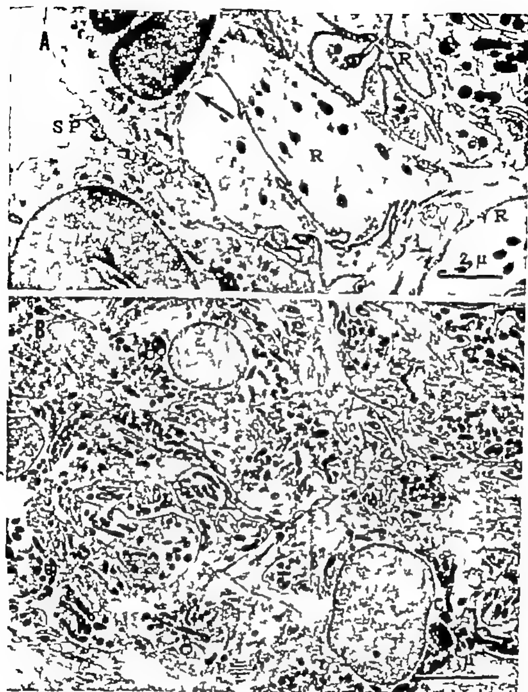


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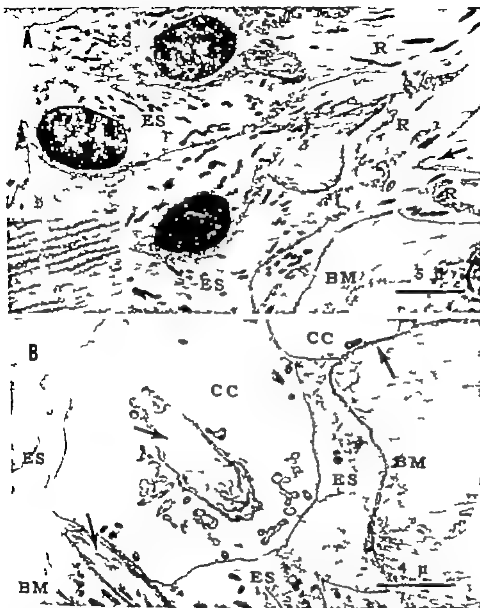


Fig. 8 Portions of the external sulcus cells (*ES*) facing the scala media.

(A) The cell surfaces are in contact with endolymph and establish an uninterrupted cell layer. The cell organelles are rich around the nucleus but the filaments (see higher magnification in insert, the diameter is 64 A) dominate the major part of cell processes or roots (*R*). Note branching of the cell processes (*arrow*) and some interdigitation of the lateral membranes. (*BM*) basilar membrane. 3500.

(B) External sulcus cells (*ES*) under the cells of Claudius (*CC*) are penetrated by the connective tissue "channel" or space (*arrows*). One of these "channels" extends deeply into the cells of Claudius. (*BM*) basilar membrane. 5000.

of the basilar membrane, then diverge into different bundles. In each bundle, cell processes interdigitate extensively but only a few macula adherens and fascia occludens are observed. The root cell processes are continuously surrounded by the basal lamina and a zone of fibrils, except where they abut the spiral prominence epithelium (Fig. 6 A). They co

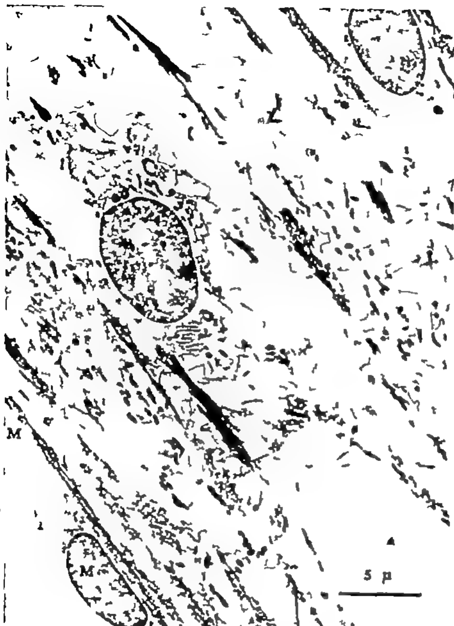


Fig 9 The spiral ligament adjacent to the scala tympani (*ST*). At the luminal surface is a discontinuous layer of mesenchymal epithelial cell (*M*) and layers of fibrils. The fibrocytes (Type II) are rich in cell organelles near the lumen, but are less in the deeper part (Type I). Fibrils are in bundle form directed toward the basilar membrane, and wide intercellular spaces are present throughout. 3900.

to the fibrocytes but contact is not observed. Their basal lamina and that of the capillary wall approximate and sometimes become continuous.

The external sulcus cells often contain numerous filaments (64 A in diameter· Fig. 8 A insert) in both fetus and adult Rhesus monkeys. Filaments are generally found around the nucleus and are present in varying amounts in the deeper cellular processes where they are longitudinally arranged but wavy and parallel. The apical and perinuclear zones are rich with parallel rough endoplasmic reticula, rosette form RNA particles and Golgi apparatus. Long mitochondria and long, stacked smooth endoplasmic reticula are found in the root portion. Vesicles are also found close to the basal lamina and also at the plasma membranes of the adjacent root cells.

Fibrils of the basilar membrane are parallel and arranged tightly. At the crista basilaris they spread in all directions, more fibrils being

directed toward the spiral prominence and behind the stria vascularis. They are approximated by other fibrils of the same size and type and crisscross. At the immediate attachment area of the basilar membrane there are no cells and a little farther laterally the space between fibrils is occupied by slender cell processes of fibrocytes, similar to those seen at the spiral prominence. These fibrils of the basilar membrane and intercellular fibrils come in contact with the basal lamina of root cells.

Spiral ligament adjacent to the scala tympani
This area is essentially composed of two types of cells: mesenchymal epithelial cells at the luminal surface and fibrocytes below (Fig. 9). In contrast to other areas of the spiral ligament, these cells and fibrils are more loosely organized and show wide intercellular spaces throughout.

The mesenchymal epithelial cells are few in comparison to the number seen at the basilar membrane. Their cell bodies are elongated but do not form a continuous layer over the connective tissue elements. Their cytoplasm is light with cellular organelles. They contain a Golgi network, some rough ER, free RNA particles, many vesicles and some intracellular filaments. The cell attachments, macula adherens, are observed frequently particularly at the corners of a few aggregated cells. The macula adherens is composed of an intermediate line and a cytoplasmic condensation at the adjacent plasma membranes (Fig. 3 D). The spaces between the mesenchymal epithelial cells are partially filled with bundles of fibrils, thus the perilymphatic space and intercellular space of the spiral ligament are in free communication. The mesenchymal epithelial cells lack the basal lamina.

Fibrocytes found near the lumen are elongated and tend to show more cytoplasmic organelles than those found in the deeper part. Some of them resemble the cells behind the spiral prominence containing parallel rough endoplasmic reticula, many mitochondria, extensive Golgi network, lysosomes and numerous vesicles. Fibrocytes in the deep zone are more loosely spread than in any other part of the spiral ligament. Fibrils are in bundle form and tend to be directed toward the basilar membrane. Blood vessels are free in wide connective tissue spaces. Their endothelial cells are surrounded by one or two layers of pericytes.

DISCUSSION

The main type of cell in the spiral ligament is the fibrocyte. The chromaffin cells described in the guinea pig by Hilding (1965) could not be found in our specimens. These cells and other types of cells, if present, would be limited in number. The stroma cells are reported in the spiral prominence of the rat by Spoendlin (1967). Our observation of the same area in the Rhesus monkey revealed compactly arranged fibrocytes rich in cell organelles. There seemed to be some morphological differences among species e.g. the fluid transport characteristics of these cells are prominent in small animals such as the bat and rat, while such evidences are not striking in the Rhesus monkey. The fibrocytes containing many cell organelles are also found adjacent to the external sulcus cells, to some extent adjacent to the scala vestibuli, and very few near the scala tympani. Their distribution near the fluid surfaces suggests a close relation to fluid metabolism. Their cytological characteristics may be influenced by the chemical composition of perilymph and/or endolymph.

In contrast to these cells near the fluid surfaces, the cells behind the stria vascularis and along the entire length of the deeper part of the spiral ligament contain relatively few cell organelles. The cell processes of a single cell show considerable morphological variations some look almost empty while others contain many mitochondria. These fibrocytes are scattered in a wide space but their long cell processes establish numerous attachments with each other. Since differences in morphology and distribution of fibrocytes are observed, we propose to classify them as Type I and Type II. The Type I fibrocytes are numerous, appear

quiescent, and are located away from the fluid *surfaces of the scalae. The Type II fibrocytes* are active and are located at the fluid surfaces of both scalae vestibuli and tympani, at the spiral prominence, and near the external sulcus cells.

The fibrocyte Type II frequently contains long mitochondria with longitudinally arranged cristae. Mitochondria with longitudinal cristae are observed in the neurons (Palay & Palade, 1955) Fawcett (1966) shows mitochondria with transverse cristae in the proximal tubule of the summer frog changing into longitudinal cristae after starvation. The presence of this type of mitochondria is consistent in the Rhesus monkey and is therefore presumed to be a normal occurrence However some of these mitochondria appear to increase in matrix *density and fuse together thus resulting in a* huge or grotesque shape They may represent a transitional stage of mitochondrial degeneration. It is interesting to note that the fibrocytes in the spiral prominence and external sulcus cell areas appear more vulnerable than those in other areas as indicated by the venous obstruction experiment of the labyrinth by Kimura & Perlman (1956)

The basal lamina is lacking below the cell along the scalae vestibuli and tympani There are numerous intercellular spaces at both of these scalae, particularly adjacent to the scala tympani suggesting that the fluid in the spiral ligament and perilymph are similar in their chemical composition. Presence of these spaces suggests further that any minute, injected substance in perilymph is likely to be found in the spiral ligament, probably more in the spiral ligament adjacent to the scala tympani. This type of finding is not necessarily related to fluid resorptive activity of the spiral ligament but could be associated more with number and size of the intercellular space The basal lamina is also lacking behind the stria vascularis. In fact, the scala media is completely surrounded by a continuous basal lamina of epithelial cells except at the stria vascularis and the habenula perforata. The junction between the stria vascularis and the spiral ligament is rather tight, while at the habenula there are intercellular spaces between the nerve fibers.

The external sulcus cells are of considerable interest recently These epithelial cells are unusual, being located below the other epithelial cells or on the fluid surface Surrounded by the basal lamina, their cytoplasmic processes divide and extend upwards even behind the mid-portion of the stria vascularis. The proposed function of these cells varies. endolymph secretion (Shambaugh Sr., 1908 Lawrence, 1956) and endolymph resorption or phagocytosis (Fleandt & Saxen, 1936 Altmann & Waltner 1947) From our ultrastructural study we cannot determine whether they are involved in secretion or absorption In agreement with *Duvall (1969) it seems certain that there is* no secretory duct, although intercellular channels have been observed in this region (Ilberg *et al.* 1968) In the Rhesus monkey long connective tissue channels between the external sulcus cells extend to the cells of Claudius but do not open directly into the endolymphatic fluid space. The physiological significance of these channels is not understood at the present time. The filamentous nature of the external sulcus cells and cellular location could be suggestive of the myoepithelium which is thought to regulate a secretory product by means of contraction (Ellis, 1965) However the presence of filaments, even in the fetus, is more indicative of a supporting nature, possibly for the spiral ligament and basilar membrane. The external sulcus cells show a close relationship to the basilar membrane, fibrocytes Type II and blood vessels. The mechanical movement of the basilar membrane could be transmitted to the external sulcus cells and may indirectly influence vascular flow through the stria vascularis and the spiral ligament, and or influence fluid transport activity of fibrocytes Type II in this area.

The intercellular fibrils are regarded as protein in nature, different from collagen and elastin (Iurato, 1962) Cigès (1965) believes that

the spiral ligament fibrils of the human are reticulin, and that age produces no changes in the ligament proper but causes diminution of the elastic stroma of the basilar membrane. Hamilton (1967) is of the opinion that the fibrils of the vestibular perilymphatic space are keratin. The fibrils of the spiral ligament of the Rhesus monkey are similar to those of the vestibular labyrinth a cross section of fibrils often shows four subfibrils. However other fibrils containing more subfibrils, diffuse form, and large oval-shaped fibrils are observed The significance of these different types of fibrils is not clear although they are presumed to be produced by the same type of fibrocytes

There are three types of cell junctions in fibrocytes: fascia occludens, fascia adherens and macula adherens. Fusion of the outer leaflets of the unit membrane was not observed in the fibrocyte of the rat inner ear (Hamilton, 1967 Iurato 1967) The presence of the zonula occludens and desmosome between the basal cells of the stria vascularis is reported by Spoendlin (1967) In our specimens, the fascia occludens is the most common type of cell junction and is observed even between the cell processes of the same cell. This tight junction may serve to retain secretory substances in the small enclosure of channels in which fibrils are formed, and also may establish a close, functional relationship between fibrocytes. The junctions between fibrocytes, between fibrocytes and basal cells, and between basal cells are similar in type, except that the latter two groups show the junctions more frequently with the macula adherens and fascia occludens alternating. They would provide strong attachments as well as a tight seal to the stria vascularis. The spiral ligament plays a major role of support for the endolymphatic epithelial lining, and also appears to take an active part in cochlear fluid metabolism.

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ZUSAMMENFASSUNG

Elektronenmikroskopische Untersuchungen des Ligamentum spirale des Rhesusaffen zeigen zwei Fibrozytentypen. Type I enthalt verhaltnismassig wenig Organellen und ist im tiefen Teil des Ligamentum spirale lokalisiert, angrenzend an die Stria vascularis. Type II enthalt viele Organellen und liegt nahe der Oberflache der Scalae vestibuli und tympani, hinter dem Epithelium des Prominentium spirale und hinter den Zellen des Sulcus externalis. Die Fibrozyten zeigen viele Bindungen zueinander (Fascia occludens, Fascia adherens und Macula adherens) und sogar zwischen den Prozessen derselben Zelle. Type II ist mit Flussigkeit Metabolismus beschaftigt und enthalt oft lange Mitochondrien mit der Lange nach angeordneten Cristae. Zwischen den Zellen des Sulcus externalis und den Zellen des Claudius befinden sich Kanale aus Bindegewebe. Es wird die physiologische Bedeutung der Zellen des Sulcus externalis diskutiert

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X RAY IRRADIATION OF THE INNER EAR OF THE GUINEA PIG

An Electron Microscopic Study of the Degenerating Outer Hair Cells of the Organ of Corti

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From the Department of Otolaryngology Rikshospitalet and the Anatomical Institute University of Oslo Oslo Norway

Received June 23, 1969

The inner ear of guinea pigs was irradiated with single doses of 7000 R X-rays. A electron microscopic study of the organ of Corti was performed after 1 / 3 4, and 6 hours.
Changes ere noticed in the outer hair cells of the tw basal coils while the inner hair cells, the outer hair cells of the two apical coils and the supporting cells ere normal.

The first changes were observed in the nuclei and consisted of clumping of the chromatin and the inter chromatinic substance. Later the nuclei became pyc notic

In the cytoplasm the early changes were: increase of peculiar elements of interconnected smooth and rough endoplasmic reticulum, various mitochondrial alterations; increase in the number of glycogen-like granules, increase in the number and size of lysosome-like bodies in the apical portions of the cells and the appearance of sequesters of small cytoplasmic areas delimited by a double or single membrane. Severely altered cells appeared shrunken.

At later stages cell debris with an electron transparent matrix and numerous vacuoles were observed.

No signs of phagocytosis were noticed. The elimination of the degenerated elements is presumably due to autolysis

In a light-microscopic study on the early degenerative changes in the organ of Corti following X-ray irradiation, changes were found in the nuclei of the outer hair cells in the basal coil three hours after the irradiation (Winther 1969 *a* and *b*). After six hours, degenerated outer hair cells were found in the same region.

The present electron microscopic investigation was undertaken in order to elucidate the nuclear alterations of the outer hair cell at the ultrastructural level and to study the cytoplasmic alterations which could not be sufficiently analyzed in the light microscope

MATERIALS AND METHODS

Fourteen guinea pigs were irradiated with single doses of 7000 R. They were sacrificed 1 /2, 3 4 and 6 hours after completion of the irradiation. The source of radiation was a Siemens Stabilipan roentgen apparatus oper ated at 290 kV 12 mA, and equipped with a Thoraeus filter II (0 8 mm Sn, 0.25 mm Cu, and 1 mm Al) Focus-skin distance was 232 mm. Dose rate at the site of the inner ear was 200 R/min. The X ray dose was delivered to the left half of the skull through a rectangular opening (20 mm × 17 mm) in a lead sheet (thickness 10.5 mm) which also shielded the rest of the animal. For further details regarding the experimental animals, the irradiation conditions, and the dosimetry the reader is referred to a previous paper (Winther 1969 *b*)

The guinea pigs were decapitated and the lower jaw the muscles on the skull base and the remaining vertebrae were removed. The temporal bones were isolated and a wide open ing was made to the vestibulum. The utriculus and the sacculus as well as the round and oval windows were opened and a hole was made in the bone at the apex of the cochlea. Within 2–3 min after the animals were sacrificed, the

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The present electron microscopic investigation was undertaken in order to elucidate the nuclear alterations of the outer hair cell at the ultrastructural level, and to study the cytoplasmic alterations which could not be sufficiently analyzed in the light microscope.

MATERIALS AND METHODS

Fourteen guinea pigs were irradiated with single doses of 7000 R. They were sacrificed 1 /₂, 3 4 and 6 hours after completion of the irradiation. The source of radiation was a Siemens Stabilipan roentgen apparatus operated at 290 kV 12 mA, and equipped with a Thoraeus filter II (0.8 mm Sn, 0.25 mm Cu, and 1 mm Al) Focus–skin distance was 32 mm. Dose rate at the site of the inner ear was 200 R/min. The X-ray dose was delivered to the left half of the skull through a rectangular opening (20 mm × 17 mm) in a lead sheet (thickness 10.5 mm) which also shielded the rest of the animal. For further details regarding the experimental animals, the irradiation conditions, and the dosimetry the reader is referred to a previous paper (Winther 1969 *b*)

The guinea pigs were decapitated and the lower jaw the muscles on the skull base and the remaining vertebrae were removed. The temporal bones were isolated and a wide opening was made to the vestibulum The utriculus and the sacculus as well as the round and oval windows were opened and a hole was made in the bone at the apex of the cochlea. Within 2–3 min after the animals were sacrificed, the

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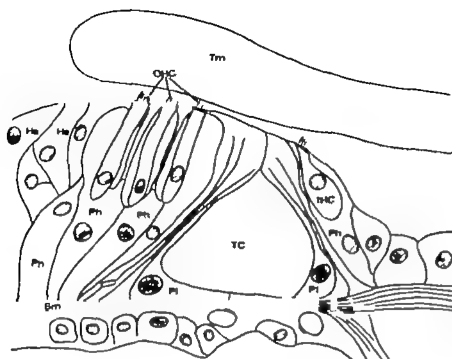


Fig 1 Schematic drawing of the organ of Corti. Outer hair cells (*OHC*); inner hair cells (*IHC*); Phalangeal cells (*Ph*); pillar cells (*Pl*); Hensen cell (*He*); Basilar membrane (*Bm*); Tectorial membrane (*Tm*); tunnel of Corti (*TC*).

labyrinth was irrigated with the fixative solution through a thin plastic catheter connected to a 5 ml syringe. The fixative used was mainly veronalacetate buffered 1.5% osmium tetroxide at a temperature of 4 C. The temporal bones were in this solution for 1–2 hours and then in physiological saline. Some of the animals were fixed for 2–3 hours in cacodylate buffered 2.5% glutaraldehyde with the same technique and after rinsing in physiological saline the material was postfixed in 1.5 osmium tetroxide for 1–2 hours.

After partial dehydration in ethanol specimens from each of the four coils were selected under a dissecting microscope. Dehydration was then completed and the specimens

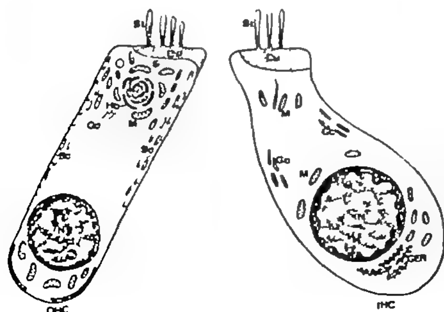


Fig 2 Diagrammatic representation of the outer (*OHC*) and inner (*IHC*) sensory cells of the organ of Corti. Nucleus (*N*); mitochondria (*M*); subsurface cisterns (*Sc*); Hensen body (*Hb*); Golgi complex (*Gc*); granular endoplasmic reticulum (*GER*); cuticular plate (*Cu*); stereocilia (*St*).



Fig. 3. Nucleus of normal outer hair cell. Fixation/staining: osmium tetroxide/lead citrate. Chromatin (*Ch*); nucleolus (*Nl*); small granules of the interchromatinic substance (*black arrow*); larger granules of the interchromatinic substance (*white arrow*); pore in the nuclear envelope (*black triangle*). 34,000.



Fig. 4. Nucleus of normal outer hair cell. Fixation/staining: Glutaraldehyde-osmium tetroxide/uranyl acetate-lead citrate. Chromatin (*Ch*); small granules of the interchromatinic substance (*black arrow*); larger granules of the interchromatinic substance (*white arrow*); pore in the nuclear envelope (*black triangle*). 34,000.



Fig 8 Apical portion of an outer hair cell 3 hours after the irradiation. Note the stack of granular endoplasmic reticulum (*GER*) continuing on both sides with array of smooth endoplasmic reticulum (*SER*) The arrows point to myelin-like structures formed by the stacks of cisterns. The cuticular plate (C) th stereocilia has normal appearance. ...000.

outer hair cells their normal ultrastructure deserves a detailed description.

It is known that the appearance of the nucleus is dependent on the fixation staining methods employed (Marinozzi & Gautier 1962) Normal and irradiated specimens were therefore investigated following two fixation staining procedures. 1 Fixation in osmium tetroxide and staining with lead citrate and – primary fixation in glutaraldehyde post-fixation in osmium tetroxide and staining with uranyl acetate and or lead citrate In agree-



Fig. 9 Detail of Fig. 8 × 39,000.

ment with Fawcett (1966) the so-called heterochromatin was found to be more heavily stained following the second procedure, but no relevant qualitative differences were found. In the micrographs of the nucleus of the outer hair cells, chromatin substance interchromatinic material, and nucleolus are distinguished. The heterochromatin is distributed all over the nucleus, mainly in isolated or interconnected masses and is particularly concentrated along the nuclear membrane (Figs. 3 and 4). The interchromatinic substance consists of numerous granules of 80 A to 120 A often grouped in a number of four to eight, a small

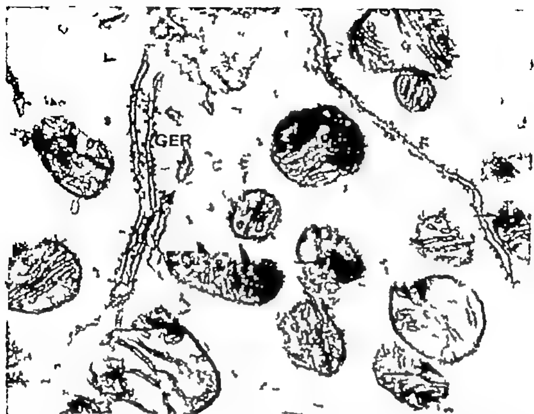


Fig 10 Mitochondria from an outer hair cell of an animal sacrificed 4 hours after the irradiation. Note the crystals (*arrows*) within the mitochondria. Granular endoplasmic reticulum (*GER*). × 52,000.

les of about 350 A, and an apparently ·s ground substance The nucleolus, ·n 1 μ in diameter is located off centre, ·d is composed of four parts as in other types of cells (Bernhard & Granboulan, 1968) The pars fibrosa, the pars granulosa, the pars amorpha, and the nucleolar associated chromatin (Fig. 3) In none of the controls did the findings differ from the normal picture

Irradiated material

No changes were noticed in the inner hair cells or the supporting cells when compared to the controls The outer hair cells of the two apical coils were usually normal also In the outer hair cells of the basal coil and sometimes in the outer hair cells of the second coil from the base of the cochlea, nuclear and cytoplasmic changes were found. To make the description of these changes easier a division into stages is adopted This subdivision is somewhat arbitrary since transitional features are encountered thus indicating that the degenerative process is a continuous phenomenon. In specimens from animals sacrificed four and six hours after the irradiation, all stages of the degeneration process were observed In specimens from animals sacrificed at one-and-a-half hours mostly nuclear changes were encountered.

Stage 1 This stage was characterized by nuclear changes without evident cytoplasmic alterations.

The chromatin was clumped in large dense masses, more conspicuous than in normal material. Also the smaller granules of the interchromatinic substance were more densely packed Large electron transparent spaces were thus created within the nucleus (Figs 5 and 6) The nucleolus was apparently normal and the nuclear envelope and the pores were as in the controls



Fig. 11. Outer hair cell 4 hours after the irradiation. The cell is collapsed, highly electron-dense and with a pycnotic nucleus (*N*). Normal appearing cuticular plate with stereocilia. Efferent nerve endings (*N*). Hensen cell (*H*). Phalangeal cells (*Ph*). 9000. Insets show (*a*) transversally sectioned degenerating outer hair cell, 9000· (*b*) degenerating outer hair cells. Phase contrast micrograph, 1350.

Stage II. The nuclear changes were of the same type as in stage one, but more pronounced and accompanied by cytoplasmic alterations.

Small separate masses of the nucleolar pars granulosa were observed, suggesting fragmentation of the nucleolus. The nuclear membrane, which otherwise appeared normal, was scalloped (Fig. 7 *a*, *b*). In the cytoplasm, conspicuous alteration of the endoplasmic reticulum was noted. Large networks of smooth endoplasmic reticulum were often found in the supranuclear part of the cell. These networks resembled to some extent the Hensen bodies but lacked the orderly arrangement. In the apical and infranuclear portions of the cell long cisterns of granular endoplasmic reticulum were observed. Frequently the cisterns were continuous with the netlike profiles of the smooth endoplasmic reticulum (Figs. 8 and 9). The cisterns of granular endoplasmic reticulum were either isolated or grouped in stacks consisting of up to eight cisterns. At places the stacks formed whorled myelin-like structures. Ribosomes were lacking at these places even if present on the rest of the cisterns.

The mitochondria were usually normal, but in a number of cells mitochondria with crystal-like inclusions were observed (Fig. 10).

Bodies, limited by a single membrane, and containing granules of various diameter and electron density were found more frequently than in the controls.

Stage III. Th nuclear and cytoplasmic changes, although more pronounced, were usu-

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AN AUDIOMETRIC SURVEY OF THE INCIDENCE AND CAUSES OF HEARING DEFECTS AMONGST DRAFTEES IN THE VASA MILITARY DISTRICT FINLAND 1954–1968

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In the years 1954 and 1968, 992 and 1155 young men (19 years of age) were examined as part of their conscription examination for hearing defects. The survey was conducted in the military district of Vasa. The examination was performed with a tone audiometer (screening within the frequency range of 250–4000 cps at an intensity level of 20 dB ISO). Hearing defects were found in 12.10 and 11.26% respectively with conductive defects 6.15 and 5 03% respectively sensorineural hearing defects 5 95 and 6.23% respectively The incidence of adhesive otitis in the two series was 1.9?% in 1954 and 1.36% in 1968. An obvious decrease of the incidence of chronic otitis media was noted, the incidence being 2.72% in 1954 against 1 13% in 1968 I 1954 lasting binaural hearing defect of at least 40 dB ISO in the better ear was found in 1.01% of those examined as against 0.51% in 1968 The cause f these lasting, bilateral, relatively bad hearing defects in 1954 in half the cases (5 out of 10) was either chronic otitis or adhesive otitis, while the rest of the cases consisted of sensorineural hearing defects: in 1968, 2 out of total of 6 cases were caused by chronic otitis or adhesive otitis.

Ear disease and hearing defects are a common cause either for the complete exemption of young men of calling-up age from military service or for their allocation to fatigue work or other duties. In the years 1929–1938 be tween 6 39 and 12.96% of all recruits in Finland were exempted already at the time of conscription because of ear disease or hearing de fects. The majority of them (3.26–10 02%) were exempted on the grounds of chronic bilateral middle-ear inflammation. Already dur ing the period 1946–1954 there seems to have occurred a considerable decrease in the incidence of hearing defects among the draftees, since at this time between 3 09 and 5 00% were exempted because of hearing defects or ear disease either at conscription or after the beginning of training. The proportion of chronic middle-ear inflammation seems also to some extent to have decreased compared with the earlier period in that between 1 08 and 2.35% of the conscripts were exempted for this reason

In order to obtain a clearer picture of the incidence of hearing defects and their aetiology amongst young men of conscript age, the author conducted mass audiometric examinations during 1954–1955 of the draftees in Nyland's and Vasa military districts (Juselius, 1962) The hearing tests, which were performed on a total of 2071 young men, were conducted between the frequencies 250–4000 cps at an intensity of 10 dB Hearing defects were detected in 11 4% sensorineural defects in 6 08% and conductive defects in 4.20% of those examined. The majority of the conductive hearing defects were caused by adhesive or chronic otitis, or as a consequence thereof. 1 79% of all those examined had uni- or bilateral adhesive otitis and ? 12% uni- or bilateral chronic middle-ear inflammation 0 63% of the exam ned h d a residual hearing defect of at least 30 dB in the better ear In more than

half of these cases (8 out of 13) the basic cause of the hearing defect was adhesive otitis or chronic middle-ear inflammation.

The results from the two military districts examined showed no significantly large differences, with the exception of the frequency of hearing defects caused by inflammation the incidence of adhesive otitis (1 92 %) and chronic otitis (2 72 %) was greater in Vasa military district than in Nyland's military district (1 67 and 1 92 % respectively) This difference was *thought to be contributed to the* fact that, at the time of the examination, there was as yet no E.N T specialist in this area, whereas most of the country's otolaryngologists had practised in Nyland's county for several decades.

The investigations conducted in 1954–1955 included young men of 19 years, representing the age-groups born 1935–1936 These groups during childhood and adolescence had possibilities for treatment of possible middle-ear inflammation with sulphonamides, which were introduced for therapeutic use at the end of the 1930s. As it would be interesting to see to which extent the incidence of inflammatory ear disease and subsequent hearing defects has decreased after the introduction of penicillin other antibiotics in therapy it was decided to conduct a new investigation on the same principles in the autumn of 1968 in connection with the conscription in the Vasa military district.

The Actual Investigation

The examination was conducted in September–October in the military district of Vasa. The substance of the investigation consisted of 1155 men, all 19 years of age and born in 1949 who according to the law must present themselves for conscription at this age.

A battery-driven, transistorized tone audiometer (Madsen Electronics, type TBN 60), equipped with noise-isolated headphones of type ME 70, was used for the examination. Before the examination, the audiometer was calibrated by means of an artificial ear

The hearing tests were conducted in a quiet room, and the so-called screening-method was used. The screening was performed within the frequency range 250–4000 cps with an intensity level of 20 dB (ISO) A camera silenta was not available, but at the times of the testings, it was quite easy to find a room where the external noise was insignificant. Each conscript examined was placed face turned away from the audiometer and was instructed to raise the ipsilateral hand when the tone signal was heard *in the same ear* In order to eliminate dissimulation, the tone signal was changed at random from one ear to the other The signals were given at different intervals and were of varying duration.

Those of the examined who perceived all tones at an intensity of 20 dB were considered to have normal hearing. A hearing defect of at least 25 dB or more for two tones in the range 250–2000 cps, or at least 30 dB for the tone in the range 4000 cps was considered pathological.

All the examined, judged by these criteria to have diminished hearing, were referred for a thorough otological examination, comprehensive speech and whispering tests, complete air conduction audiogram within the tone range 250–8000 cps and bone conduction audiogram within the tone range 250–4000 cps. Tympanic membrane examination involved the routine procedure, and any suspicion of an adhesive process was further investigated with a Brünings Siegle's pneumatic ear speculum. If *adhesive otitis or tubal occlusion* was suspected, tubal cathererization was performed, after which the hearing function was re-assessed with the audiometer A tuning fork test was used when necessary

RESULTS

The overall incidence of hearing defects among conscripts is illustrated in Table 1 For comparative purposes, the results from the earlier examination in the same military district in 1954 are also shown in the same table. Since

the audiometer used for the tests in 1954 had been adjusted to a different calibration standard (ASA 1951), the results from the earlier examination were converted to correspond with the calibration standard of the transistorized audiometer (ISO 1964). All decibel values that hereafter are demonstrated in the text and tables refer to ISO 1964 values, which are on average approximately 10 dB higher than the corresponding ASA 1951 values (Davis, 1965)

Table 1 *Incidence of audiometrically diagnosed monaural or binaural hearing defect among draftees in the Military District of Vasa Finland 1954 and 1968*

Year	No. tested	Total no. of men with hearing defect	Men with monaural hearing defect	Men with binaural hearing defect
1954	992	120 (12.10)	69 (6.96)	51 (5.14)
1968	1155	130 (11.26 %)	98 (8.48)	32 (2.77)

DIAGNOSES

To the diagnostic group of *otosalpingitis* have been included all cases of tubal occlusion, where with the help of tubal catheterization, with or without paracentesis, the hearing function has been restored to normal levels or considerably improved. The diagnostic group thus only includes cases where the prognosis with regard to the possibilities of restoring the hearing has been considered good.

Otitis media adhesiva chronica

In this diagnostic group have been included cases where, with the help of Brünings-Siegle's speculum, obvious verifiable adhesive processes have existed, and tubal catheterization, no or a very insignificant improvement in the hearing was achieved, although air was able to pass into the middle ear

Otitis media suppurativa chronica et residua

This group comprised cases with a continuing chronic suppurative process in the middle ear and cases of dry drum membrane defect or sequelae following operation for chronic suppuration.

Hypacusis conductiva e causa ignota

Within this diagnostic group have been included cases with diminished hearing of conductive type, that could not be classified under the aforementioned diagnostic groups.

Hypacusis perceptiva

Within this group have been included cases with normal ear drum findings but diminished hearing of sensorineural type. If signs of sensorineural hearing defect have appeared in connection with an obvious adhesive process or chronic suppuration in the middle ear the perception injury has been considered secondary and these cases have been included under the general heading of conductive hearing defects. In this group there is a relatively large number of isolated hearing defects of c^5dip type.

Hearing defects that have been caused by

Table 2. *Causes and incidence of lasting monaural and binaural hearing defect among National Servicemen in the Military District of Vasa Finland, 1954 and 1968*

	Otitis media adhesiva chronica	Otitis media suppurativa chronica	Hypacusis conductiva causa ignota	Hypacusis perceptiva
1954 (No. tested, 992)				
Men with lasting monaural or binaural hearing defect	19 (1.92)	27 (2.72)	1 (0.10)	59 (5.95 %)
1968 (No. tested, 1155)				
Men with lasting monaural or binaural hearing defect	18 (1.56 %)	13 (1.13)	1 (0.09 %)	72 (6.23 %)

Table 3 *Causes and incidence of lasting binaural hearing defect among National Servicemen in the Military District of Vasa Finland 1954 and 1968*

	Otitis media adhaesiva chronica	Otitis media suppurativa chronica	Hypacusis conductiva e causa ignota	Hypacusis perceptiva
1954 (No. tested. 992)				
Men with lasting binaural hearing defect	7 (0.71 %)	9 (0.91 %)	1 (0 10 %)	26 (2.60 %)
1968 (No. tested 1155)				
Men with lasting binaural hearing defect	1 (0.09 %)	4 (0.35 %)		18 (1.56)

otitis media adhesiva chronica otitis media suppurativa chronica et residua, hypacusis conductiva e causa ignota and *hypacusis perceptiva* have been considered as lasting. Here the possibility of improvement by means of operation or hearing aid has not been taken into account.

The causes of the lasting hearing defects are analysed in Table 2, which also demonstrates the interrelationships between the two examinations conducted in 1954 and 1968.

In Table 3 the causes of the bilateral lasting hearing defects detected at both examinations are illustrated.

Table 4 shows the incidence of different de s of lasting bilateral hearing defects in young conscripts. The analysis has been made according to the degree of the diminished hearing of the better ear within the tone range 250–4000 cps.

In Table 5 a correlation is drawn from the examinations in 1954 and 1968 of the cases of lasting, relatively high-degree binaural hearing defects. The table shows diminished hearing in decibels (ISO) within the tone range 250–4000 cps, as well as within the so-called most important speech area, namely 500–2000 cps. The hearing loss of both the better and the worse ear as well as the nature of the hearing defect, are demonstrated in the table.

Table 4 *Incidence of lasting binaural hearing defect of varying degree among National Service men in the Military District of Vasa, Finland 1954 and 1968*

Classified according to hearing loss in the better ear within the frequency range 250–4000 cps

Year	No tested	< 40 dB (ISO)	40–70 dB (ISO)	> 70 dB (ISO)
1954	992	33 (3.33)	8 (0.81)	2 (0.20)
1968	1155	17 (1 47 %)	5 (0.43)	1 (0.09)

DISCUSSION

A comparison of the results from the examinations in 1954 and 1968 in the military district of Vasa clearly shows that the incidence of chronic middle-ear inflammation and sequelae have considerably decreased. In 1954 chronic middle-ear inflammation or after effects thereof were found in 2 72 % of those examined, as compared with 1 13 % in 1968 The introduction of antibiotics in therapy has obviously contributed to this decrease, but one must also include the effect of the raised standard of living and better public health. At the time of the first examination there was no E.N T specialist in the whole of the county of Vasa, the Central Hospital in Vasa came into existence in 1956 and it is only from the time that a specialist ear nose and throat department has been available Thus the better possibilities for diagnosis and treatment have surely played a considerable part in the decreased incidence shown from the comparison of these series.

Table 5 *Men with lasting relatively severe deafness Degree and cause of deafness*

	Hearing loss within frequency range 250–4000 cps, in dB (ISO)	Hearing loss within frequency range 500–2000 cps, in dB (ISO)	Diagnosis
1954			
Better ear	48	49.3	Hypacusis perceptiva
Worse ear	48	49.3	
Better ear	50	51	
Worse ear	54	51	
Better ear	51	51	
Worse ear	58	59.3	
Better ear	41	42.6	
Worse ear	53	56	
Better ear	40	42.6	
Worse ear	41	42.6	
Better ear	43	42.6	Otitis media suppurativa chronica
Worse ear	73	76	
Better ear	51	56	Status ex operatione radicali auris mediae
Worse ear	58	61	
Better ear	72	76	Residua post otitim cum perforatione persistente membranae tympani Status ex operatione radicali uris mediae
Worse ear	75	79.3	
Better ear	78	77.6	Residua post otitim cum perforatione persistente membranae tympani Otitis media suppurativa chronica
Worse ear	88	91	
Better ear	40	46	Residua post otitim cum perforatione persistente membranae tympani
Worse ear	54	54.3	
1968			
Better ear	63	60	Hypacusis perceptiva
Worse ear	63	61.6	
Better ear	63	70	
Worse ear	65	70	
Better ear	85	90	
Worse ear	90	93	
Better ear	51	50	
Worse ear	59	60	
Better ear	64	60	Otitis media suppurativa chronica
Worse ear	85	83.3	
Better ear	44	46.6	Residua post otitim cum perforatione persistente membranae tympani Otitis media adhaesiva chronica
Worse ear	54	48.3	

The incidence of adhesive otitis seems to be about the same in both the groups, 1.92% and 1.56% respectively. The most probable explanation for this could be that the basic aetiology stemmed from untreated otosalpingitis in childhood.

The incidence of chronic binaural middle-ear inflammation also shows a satisfactory and distinct decrease from 0.91% to 0.35% in the two investigations. This disease seems nowadays to play a considerably smaller part than previously as a cause of lasting severe loss of hearing function. In 1954 the examination revealed that half of the cases (5 out of 10) with lasting bilateral hearing defects of at least 40 dB were caused by inflammatory ear disease, whereas in the 1968 examination only 2 out of a total of [illegible] cases were of an inflammatory aetiology.

In about 6% of all those examined in the two age groups sensorineural hearing loss was found. Out of the 131 young men with sensorineural hearing [illegible]s, 53 had worked in noisy jobs. It is possible that most of the sensori-

neural hearing defects in these 53 were in fact noise injuries Through intensified information and noise prophylaxis, the incidence of such hearing injuries could be reduced to some extent.

The quite high incidence of sensorineural hearing defects amongst the conscripts emphasizes the fact that it would be desirable to examine the servicemen's hearing with an audiometer as extensively as possible either at the conscription or at the start of their military service. There is always a certain risk that a sensorineural hearing defect is worsened as a result of acoustic trauma during military service.

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ZUSAMMENFASSUNG

In den Jahren 1954 und 1958 wurde in dem Militärbezirk von Vasa bei der Einberufung zum Heeresdienst das Gehör von 992 bzw 1155 Wehrpflichtigen (Alter 19) geprüft. Die Gehöruntersuchungen wurde *audiometrisch durchgeführt* (Frequenzbereich 250-4000 Hz bei einer Intensität von 20 dB ISO). Hördefekte wurden bei 12,10 bzw 11,26% festgestellt, Schalleitungsschwerhörigkeit bei 6,15 bzw 5,03% und Perzeptionsschwerhörigkeit bei 5,95 bzw 6,23% Die Frequenz der Adhäsivprozesse in den beiden Materialien war 1,92 bzw 1,56% Eine deutliche Abnahme in der Frequenz chronischer Otitis media konnte festgestellt werden; im Jahre 1954 war die Frequenz 2,72% gegenüber 1 13% im Jahre 1968. Im Jahre 1954 wurde bei 1,01% von den Untersuchten ei bestehender doppelseitiger Hörverlust von wenigstens 40 dB ISO des besseren Ohres festgestellt, gegen 0,51% im Jahre 1968 Die Ursache dieser bestehenden, doppelseitigen, ziemlich schweren Hördefekte war im Jahre 1954 in der Hälfte der Fälle (5 von 10) entweder chronische Otitis media oder Adhäsiv-Otitis, die übrigen Fälle dagegen waren sensorineurale Hördefekte im Jahre 1968 wurden 2 von insgesamt 6 Fällen durch chronische Otitis oder Adhäsivprozesse verursacht.

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CAN HEARING AIDS DAMAGE HEARING?

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A case report of permanent perceptive acoustic trauma following the provision of hearing aid.

For over a quarter of a century there has been concern over the possible harmful effects of amplification on hearing. Holmgren (1940) strongly affirmed that in his experience amplification never adversly affected hearing and he went on to say that in many cases the application of a hearing aid had an improving effect on residual hearing. His views were supported by Whetnall (1964) A recent World Health Organization report on the Early Detection and Treatment of Handicapping Defects in Children (1967) stated it was formerly believed that the use of an aid might cause hearing to deteriorate, but there is no evidence whatsoever to support this suggestion" Littler & Rice (1964) after 3 years experimental work on hearing aid design considered that there was ample evidence to show that continued use of sound pressure levels above 125 decibels may cause further deterioration of hearing. Barr & Wedenberg (1965) followed up 84 children with bilateral perceptive deafness until they were 15 years old, during which time all wore hearing aids. They found that none of the children whose deafness was due to rubella or neonatal factors showed any progression of hearing loss, but all those whose loss was due to meningitis, and over half those who had an hereditary loss showed spontaneous progression. They concluded that progressive loss was probably due to hereditary factors.

The study of the possible adverse effects of amplification on hearing of children appears to be hampered by two main difficulties—firstly the diagnosis of deafness may be made, and amplification provided, before an accurate audiogram can be obtained, and secondly in some cases there may well be a spontaneous deterioration of hearing. It therefore follows that this problem can only be studied in cases where reliable audiograms are available before amplification is begun, and where amplification has been provided in one ear only so that the unaided ear can be used as a control.

Between August 1965 and August 1968 I examined 278 children with perceptive deafness at a regional audiology centre in South Wales. All the children were reviewed approximately three times a year and all wore hearing aids. During this period a deterioration of hearing was recorded in 16 (6%) of whom 6 had sibs with perceptive deafness and a further 3 gave a history of perceptive deafness in a close member of the family The high prevalence of positive family histories of deafness and the absence of any potential aetiological factors in the obstetric, neonatal and past medical histories of these children strongly suggested that the observed deterioration of hearing was due to hereditary factors. These findings support those of Barr & Wedenberg (1965)

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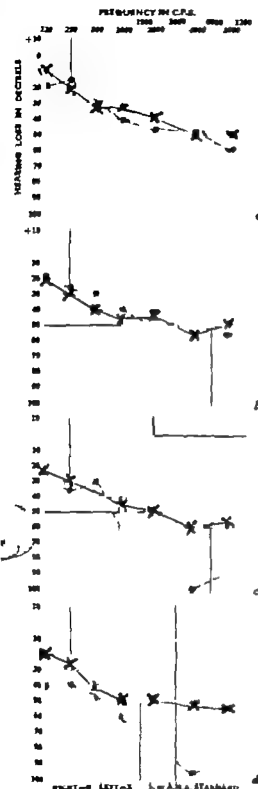


Fig. 1. (*a*) Date of test, Mar. 1965. (*b*) date of test, Sept. 1965. (*c*) date of test, Nov. 1967. (*d*) date of test, Jan. 1969.

A base line pre amplification pure tone audiogram was not available for 13 of these children since the deafness had been diagnosed before 3–4 years of age. Serial audiometry in the remaining 3 children showed evidence of deterioration of hearing in the aided ear in all 3 cases. The case report of one of these is described briefly below.

CASE REPORT

John's language development during infancy and childhood was considered normal and at 3 years he spoke using 3–4 word sentences, but with defective pronunciation. At that time there was no family history of deafness, and John's obstetric, neonatal and past medical history were normal, but his parents had begun to suspect he had difficulty in hearing. At the age of five years (March, 1965) an audiometric examination at the local authority clinic revealed a *gently sloping high frequency* perceptive deafness (Fig. 1 *a*). He was referred for specialist examination in September 1965 when the diagnosis and degree of hearing loss were confirmed (Fig. 1 *b*). Ear level amplification (right ear) was prescribed in February 1966. An audiogram in November 1967 (Fig. 1 *c*) showed severe deterioration of hearing in the right ear, most marked at 4000 cycles per second—the classical C5 dip of acoustic trauma. In December 1967 the aid was discontinued but follow up audiometry in January 1969 (Fig. 1 *d*) showed no improvement in the hearing loss. The acoustic trauma appeared to be permanent. It will be seen that from March 1965 to January 1969 there was only a 10 decibel deterioration of the hearing in the control ear (left), and this occurred over the whole of the frequency range (unlike the 50 decibel *high frequency* deterioration found in the aided ear).

In December 1967 John's younger sister was also found to have perceptive deafness similar in degree to that shown in the first audiogram in Fig. 1. Over the next 14 months her hearing showed a 5 decibel deterioration throughout the whole frequency range, in the absence of

amplification. This finding confirms the diagnosis of progressive hereditary perceptive deafness.

CONCLUSION

Evidence is presented which suggests that the use of amplification in children with progressive hereditary perceptive deafness may cause acoustic trauma and thus accelerate the hearing deterioration in the amplified ear. Fortunately this condition is uncommon (probably less than 10% of all childhood perceptive hearing losses) but nevertheless it is essential to identify this type of hearing loss before deciding to provide amplification. The presence of any or some of the following features may indicate that progressive hereditary perceptive deafness is present—a family history of perceptive deafness, the discovery in childhood, of a gently sloping 40–50 decibel perceptive hearing loss, a history of a reasonably normal speech and language development in early childhood (suggesting that hearing was either normal or minimally affected during this period), and finally a history or record of deterioration of hearing over the previous 2 or 3 years.

ZUSAMMENFASSUNG

Es wird über einen Fall von dauernder neurogener Hörwahrnehmungsschädigung als Folge der Benutzung eines Hörapparates berichtet.

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COCHLEO-SACCULAR DEGENERATION IN HEDLUND WHITE MINK

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Inner ear histological abnormalities can be recognized three weeks after birth in deaf Hedlund mink. Nuclear swelling and cytoplasmic vacuolization of cochlear hair cells, rupture of the saccular wall, and atrophy of the stria vascularis were observed beginning three weeks after birth. Secretory and sensory epithelia of the utricle and ampullae were normal. Only minimal changes were found in the lining of the endolymphatic duct and sac.

Saunders (1965) first described collapse of scala media with degeneration of the organ of Corti in Hedlund white mink. Hilding *et al* (1967) published a preliminary electron microscopic study of otological findings in Hedlund mink. They stressed abnormalcy of the stria vascularis as an important early finding, and postulated that the deafness of Hedlund mink resulted from inability of the stria to produce normal endolymph

Schuknecht *et al* (1965) have recently reviewed the many kinds of deafness that eventually manifest the histopathological findings of "cochleo-saccular degeneration" They discussed several species, including dogs, cats, mice and human. Part of the picture of cochleo-saccular degeneration in several animals is collapse of scala media with grossly inadequate volume of endolymph. Waltzing mice were discussed by van Lennep (1910) who was probably the first to attribute deafness to atrophy of the stria vascularis Waltzing guinea pigs (Lurie 1939) albino cats (Wilson & Kane, 1959) and Dalmatian dogs (Hudson & Ruben, 1962) lack endolymph volume Shaker 1 mice represent a strain of animals with hereditary cochleo-saccular degeneration which does not exhibit collapse of scala media (Kikuchi & Hilding, 1965)

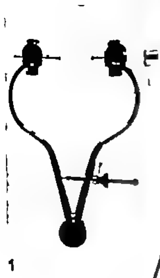
Guild (1927) performed a series of Prussian blue injection experiments which provided convincing evidence that cochlear endolymph is absorbed in the endolymphatic sac. His results tend to support the concept that endolymph is produced by the stria vascularis. A number of workers have subsequently come to the same conclusions: Yamakawa, (1929), Shirai (1942) Secretan (1944) Anderson (1948) and Engström & Hjorth (1951)

The purpose of this report is to describe our light and electron microscopic findings in Hedlund white mink at various stages after birth Our results from study of the organ of Corti, saccule, utricle and semicircular canals are included. A subsequent paper will describe circulatory abnormalities of the stria vascularis in this animal (Sugiura & Hilding, 1969).

MATERIALS AND METHODS

Mr Brownie Stula of Colchester Connecticut supplied us with normal-hearing, dark-pelted mink and with homozygous Hedlund white mink. We studied twelve normal and twelve

This work was supported by a grant from the Deafness Research Foundation, and grant 3995 from the National Institute of Neurological Diseases and Blindness.



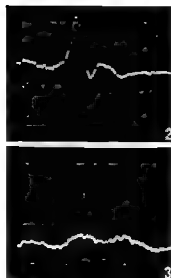


Fig 1 Adjustable headholder with hollow ear bars. Clicks were delivered from the hearing aid transducer through the hollow bars to the mink ear By appropriate adjustment of caliper and sliding bars, a good fit could be obtained at every stage of development from newborn to adult.

Fig 2 Normal round window responses as recorded from normal animal 4 weeks after birth.

Fig 3 Hedlund mink at 4 weeks. No electrical response could be demonstrated in Hedlund mink at any stage comparable to the normal one of Fig. 2.

Hedlund immature animals at various stages from newborn to six months Eight adult Hedlund and six adult normal mink, were used.

Hearing acuity of each animal was tested by startle response to hand claps and whistles. Electrical responses from the round window were recorded under general ether anesthesia in representative animals. Clicks were presented through a special adjustable headholder with hollow ear bars (Fig. 1) A hearing-aid speaker was the sound source, the clicks were generated by Tektronix pulse and wave form generators. Round window responses were amplified by RM122 preamplifiers and exhibited on a Tektronix 564 oscilloscope with storage screen. A Polaroid back was used with C 27 camera to record responses. A small, special purpose computer (CAT) was used to summate round window responses from two normal and three Hedlund mink.

After recording was completed, the animals were decapitated, and their temporal bones were quickly placed in buffered 3% cold glutaraldehyde Glutaraldehyde was perfused with small glass pipettes through the round and oval windows after stapedectomy Each part of the labyrinth was dissected separate in this solution. For investigation of the endolymphatic duct and sac, vital perfusion through the heart was used. After washing, all specimens were postosmicated for one hour Dehydration was followed by imbedding in Epon 812. Sections were cut for light microscopy at 1 0 μ and for electron microscopy at 300–600 Å with glass and diamond knives on an LKB Ultratome Contrast for the electron microscope was enhanced with uranyl acetate stain-



Fig 4 Outer hair cell (*HC*) of Hedlund mink at 3 weeks. Large clear vacuoles were found in the cytoplasm. × 2460.

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MATERIALS AND METHODS

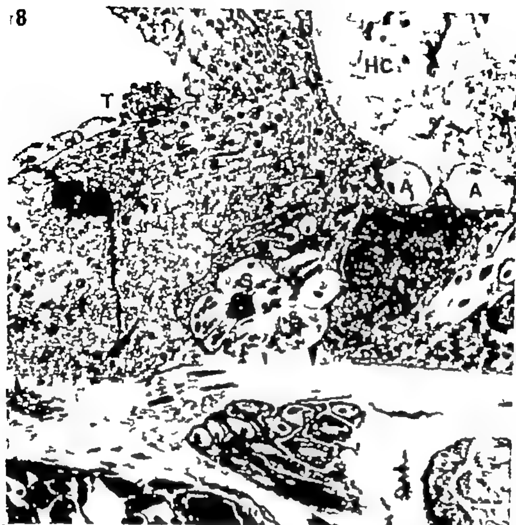
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Fig. 6. By 5 weeks, Reissner' membrane (*R*) drops close to the organ of Corti and hair cells (*HC*) f Hedlund mink. Cytoplasmic vacuolization of hair cells is more obvious. 4200.

Fig. 7. Higher magnification view of nerve endings t base of degenerating outer hair cell f Fig. 7 An afferent (*A*) and an efferent (*E*) ending are sh 23,000.



8 Inner hair cell region, 5 week Hedlund mink. ne structures of this area are morphologically nor mal. Nerve fibers (*F*) with immature Schwann sheaths are passing through the habenula perforata. The tunnel space has opened exposing tunnel crossing (*T*) and inner tunnel spiral bundle (*S*). Afferent endings (*A*) are seen at the base of an inner cell (*HC*) 3900.

(Friedmann *et al* 1965) were often found in the upper portion of hair cells as was previously reported (Hilding *et al.* 1967) Both afferent and efferent nerve endings of outer hair cells appear nearly normal at this and subsequent stages (Fig. 7) Normal appearing inner hair cells nerve fibers and endings at 5 weeks are demonstrated in Fig. 8 Tunnel fibers are also evident. Abnormal hair cells were found earliest in the basal turn. Degenerative changes of the organ of Corti appeared later in upper turns.

Our findings in the stria vascularis will be described completely in another report (Sugiura & Hilding, 1969) The changes of the strial epithelium tend to chronologically parallel those of the organ of Corti. At 2 or 3 weeks, the cytoplasm of marginal cells of the basal turn has a reduced content of mitochondria. Later as more apical turns begin to show degeneration of the stria, the epithelium of the basal turn becomes atrophic. Measurements showed a reduction to about 70% of normal width and thickness.

The endolymphatic layer of Reissner's membrane is somewhat thinner with fewer microvilli than normal by 3 weeks, but the perilymphatic layer develops cytoplasmic redund-



Fig. 9. Atrophying stria vascularis of 5 week Hedlund. Reissner' membrane (*R*) contacts the marginal cell (*M*) layer. Within the endolymphatic (*E*) layer of Reissner's are opaque granules 2 μ in diameter 4200.

ancies (Fig. 10). Microvilli are scarce on the endolymphatic surface of Reissner's membrane in adults. Often the perilympatic layer separates and otoconia are sometimes found between layers (Fig. 12). At 5 weeks, no endolymphatic space can be found between stria vascularis and Reissner's membrane (Fig. 9). Peculiar opaque granules about 2 μ in diameter were found in the endolymphatic layer of Reissner's membrane. Marginal cells of the stria lack their normal complement of mitochondria.

Large defects of the saccule wall could be demonstrated as early as 3 weeks after birth by light microscopy (Fig. 13). The cells of the sac wall were swollen with loss of limiting membranes. Often, otoconia were missing from the lumen of the saccule. Electron microscopy of 6 weeks specimens showed thickening of the saccule wall with accumulation of non-particulate debris in the small collapsed space between the wall and sensory epithelium (Fig. 14). The endolymphatic layer of the saccule wall developed cytoplasmic folding and processes similar to secretory epithelia elsewhere. In adult specimens there is a pronounced degree of this abortive conversion to secretory-type tissue (Fig. 15). The sac wall was folded upon itself in all adult specimens. Endolymphatic surfaces are adherent in Fig. 15 and the perilymphatic layer is closest to the sensory cells. Discontinuity of the sac is obviously a



Fig 10 Reissner's membrane of Hedlund at 3 weeks. As compared to normal (Fig. 11), the endolymphatic (*E*) layer is thinner and has fewer microvilli. The perilymphatic (*P*) layer has more folds and processes normal. 5600

Fig. 11 Normal mink Reissner membrane 3 weeks after birth. Microvilli cover the endolymphatic (*E*) surface. A basement membrane (*BM*) lies under the endolymphatic layer and there are relatively large openings between perilymphatic layer cells (arrow). 5600

"prerequisite in order for the "wrong side" of its wall to face the hair cells

Light and electron microscopy of the endolymphatic duct showed nearly normal findings in Hedlund mink. In the intermediate or "rugous" portion of the sac the lining cells were somewhat less well-endowed with microvilli and cytoplasmic folds than normal

The areas of secretory epithelium on the slope of the cristae had normal appearing cells by electron microscopy

DISCUSSION

Auditory function normally becomes apparent in mink [illegible] weeks or more after birth. Most of the animals studied at 2 weeks in our previous study (Hilding *et al.* 1967) gave auditory startle responses, but maturation rates vary and most in our present series did not give reproducible responses until 3 weeks after birth. With our recording technique eighth nerve responses were demonstrated with aid of summation at 3 weeks and directly at 4 weeks.

By 3 weeks, the tunnel of Corti is open in most turns (Fig. 1) and cochlear maturation is nearly complete. Efferent and afferent innervation can be demonstrated. Thus, normal mink seem to follow the pattern first described by Wada (1923) of most animals by almost completing histological maturation of the cochlea before significant auditory function appears.



Fig. 12. Otoconia (*O*) were observed in Reissner' membrane in adult Hedlund mink. Characteristically the bulk of each otoconium is missing from this section because decalcification was not performed. The otoconia have been partly engulfed by perilymphatic (*P*) layer cells (*P*). A mass of homogenous debris (*D*) occupies the space between endolymphatic layer cells (*E*) and the stria. 9800.

There was no evidence of auditory function in Hedlund mink at any stage as judged by startle response or electrical recording, including computer-summated. Other strains of hereditarily deaf animals have early periods of fairly good hearing. Evidence of temporary hearing has been particularly well documented in shaker 1 mice by Mikaelian & Ruben (1964).

Subtle hair cell abnormalities first become apparent by 3 weeks in the organ of Corti by electron microscopy in Hedlund mink. At this stage, normal mink regularly have auditory responses. It seems likely that vacuolization of the cytoplasm and nuclear swelling are merely morphological manifestations of earlier major physiological inadequacies. These early histological changes may reflect a grossly abnormal fluid environment. Either toxic mixtures of perilymph through the saccular fistula or inadequate provisioning from the stria may account for the lack of hair cell function at this and subsequent stages.

Rupture of the saccule with subsequent collapse is a relatively early finding in Hedlund mink. A major tear was demonstrated in the wall opposite the sensory epithelium in a 3 week specimen. (Our technique involved removal of the saccule after fixation instead of bony decalcification. The observed tearing was probably not artefactual because nothing similar was seen in control animals and because antemortem cytological abnormalities were obvious in the cells bordering the tear.) Later specimens showed folding of the saccule wall with two or more layers attached to each other

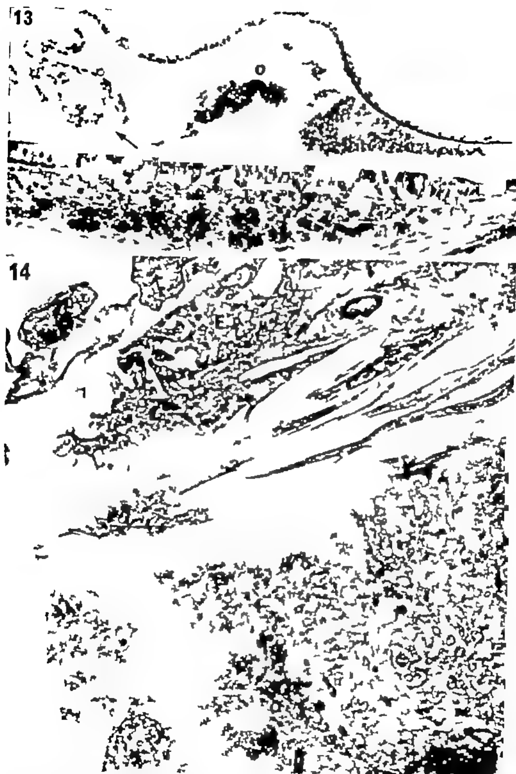


Fig 13 Collapsing ruptured saccule 3 week Hedlund mink. Within the lumen, detached otoconia (*O*) are seen. The saccule wall, near the rupture (*arrow*), is somewhat swollen. The sensory epithelium is normal at this stage. 150.

Fig 14 Saccule, 6 week Hedlund Debris (*D*) fills the greatly reduced lumen Large empty spaces in debris (*D*) may represent cholesterol or similar material dissolved during processing. The endolymphatic layer (*E*) of the opposite wall f the saccule has developed folds and processes suggesting that it has been converted to secretory function. No otoconia were found in the lumen. 5600

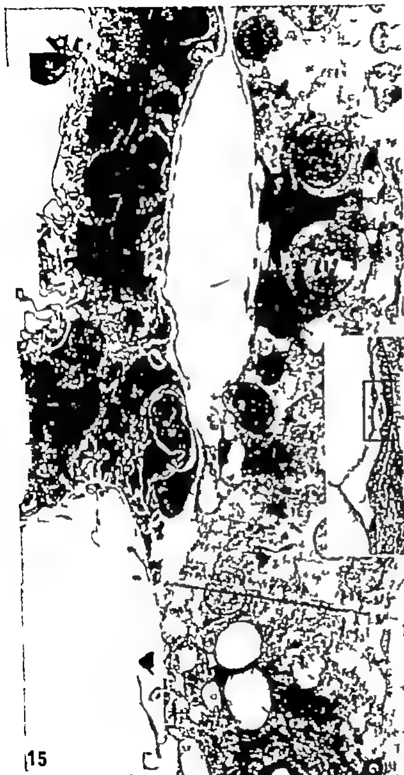


Fig 15. Adult Hedlund saccule composite of seven electron micrographs. *Inset* is light micrograph at low magnification of same specimen. The sensory epithelium is degenerated. The opposite wall has folded upon itself and fused. The perilymphatic layer of the wall faces towards the sensory epithelium. The endolymphatic layer has thickened and developed foldings to make identical with the fluid-secreting epithelium which is normally found in the slope of the crista.

The perilymphatic surface was observed within the lumen" of the saccule facing the sensory epithelium

Subsequent to the 3 week stage hypertrophy of the sac wall cells was observed. Redundant cytoplasmic folds were formed in an apparent effort to form a secretory epithelium Otoconia were found within the lumen of the saccule until about 8 months. Afterwards, they were absent in the saccule However otoconia were observed trapped in the perilymphatic layer of Reissner's membrane, suggesting that they had escaped from the saccule into perilymph and had drifted into the cochlea.

Schuknecht & Seifi (1963) described the effects of fistulous connections between endolymph and perilymph. Small tears of Reissner's membrane caused local damage. Duvall & Rhodes (1967) also found localized damage to cochlear hair cells from small experimental tears of Reissner's membrane. Igarashi (1965) showed that extensive damage to the saccule failed to interfere significantly with hearing in a series of experimental lesions of squirrel monkeys. In Hedlund mink saccular rupture combines with inadequate production of endolymph by an atrophic stria vascularis. The may have a deleterious effect.

Sagging of Reissner's membrane became ap- after 3 weeks This could be attributed to a massive endolymph leak from the saccule. However atrophic changes of the stria vascularis are noticeable by this stage and become more obvious By the adult stage, the stria is only half the width and two-thirds the thickness of normal. Ultrastructural abnormalities of the stria become more marked as maturation proceeds. Mitochondria are sparse and other cytoplasmic elements disappear As the adult stage is approached, and Reissner's membrane rests against the stria, endolymphatic secretion by the stria vascularis has obviously ceased.

ZUSAMMENFASSUNG

Histologische Fehlbildungen des inneren Ohres können drei Wochen nach der Geburt von tauben Hedlund-Nerzen erkannt werden. Kernschwellung und protoplasmische Vakuolisierung in den Haarzellen der Cochlea, Zerreissung der Wand des Sacculus, und Atrophie der Stria Vascularis wurden ab drei Wochen bemerkt. Sekretorische und sensorische Epithelgewebe des Utriculus und der Gehörampullen waren normal. Nur minimale Veränderungen wurden in der Bekleidung des Ductus endolymphaceus und des Saccus endolymphaceus gefunden.

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BIOCHEMICAL STUDIES OF OTOSCLEROSIS

Inorganic constituents by neutron activation analysis

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Neutron activation analysis was used to determine the inorganic constituents of stapes and cortical bone Zinc, sodium, magnesium, calcium and phosphorus were detected. The calcium and phosphorus concentrations in stapes and cortical bone from individuals with otosclerosis was compared to that of bone from non-otosclerotic controls. No significant differences were observed.

Etiological factors considered responsible for the development of otosclerosis have been reviewed previously (Altmann, 1962 Sorfer *et al* 1965) Studies made on the inorganic constitutents in otosclerosis have been limited to calcium and phosphorus levels in sera (Fowler 1948 Seiferth, 1937 Breytmann, 1933 Ris- 1949) and more recently a report on calcium and phosphorus concentrations in osseous tissues was presented by Maurer (1961/1962 and 1967)

In pursuance of aberrations and underlying factors present in otosclerosis the inorganic elements in osseous tissues must be investigated. Due to the limited size and quantity of material from individuals with otosclerosis and from non-otosclerotic controls the use of classical analytic procedures are impractical if not impossible. *In order to overcome these limitations*, neutron activation analysis was used in this investigation for the analysis of inorganic constituents. Unlike more conventional chemical analyses, neutron activation analysis can be accomplished on microscopic quantities without handling losses.

Neutron Activation has been used to determine inorganic constituents from various tissues and fluids in the range of 0 1 to 100 mg per sample (Ogborn *et al* 1961 Hall, 1954 Kruger & Gruverman, 1962 Samsahl & Soremark, 1961 Sato & Norris, 1950 Benson 1959 Helwig *et al* 1954 Nakyama & Blomstrand 1961 Reiffel & Stowe 1957) Additional advantages of neutron activation analyses are high precision and excellent accuracy no sample preparation is necessary it is nondestructive and it is quantitative as well as qualitative Because of these advantages, neutron activation was used to compare the inorganic constituents of bone from otosclerotic individuals with bone from non-otosclerotic controls.

PROCEDURE

In neutron activation analysis the sample under investigation is irradiated with neutrons from either a nuclear reactor or a neutron generator

[1] Deceased.

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The elements in the irradiated sample acquire an energy characteristic of a specific unstable isotope. The induced radioactivity of the sample is analyzed to determine the elements present in the sample. The quantity of each element present is determined by the amount of radioactivity which is directly proportional to the quantity of each element.

Samples

Stapes and cortical bone samples were obtained from the same individual. Eight stapedes were obtained from individuals with otosclerotic ankylosis; eight non-otosclerotic stapedes were obtained at autopsy as controls. Five cortical bone samples were obtained from five of the patients with otosclerotic ankylosis of the stapes, and two, from the non-otosclerotic controls. Cortical bone samples were removed from the posterior wall of the external auditory meatus. All samples were placed in clean glass bottles immediately upon removal from the patient. After obtaining all twenty-three samples, they were dried to constant weight and then placed in their own individual cellulose capsules (size number two) which were in turn placed in polyethylene vials (two cm^3) for irradiation. The osseous tissues were analyzed for their inorganic constituents by neutron activation analysis.

Irradiation of samples

The ten megawatt nuclear reactor of the Air Force Nuclear Engineering Center (AF NEC) at Wright Patterson Air Force Base, Ohio, was used to irradiate the samples. The pneumatic transfer system at the AF NEC was used to expose each individual sample for ten minutes to a neutron flux of 5×10^{13} neutrons per square centimeters per second (n/cm^2 sec). A known quantity of calcium carbonate to be used as a comparative standard for the determination of calcium was similarly irradiated in the pneumatic transfer system for ten minutes.

Each sample, as well as the calcium carbonate comparative standard, was irradiated along with an aluminum-cobalt alloy wire flux monitor (0.483% cobalt). These flux monitors were used to correct for any variation in neutron flux during the irradiation of each sample and standard.

Quantitative determinations of calcium & phosphorus in samples

Calcium. Within two minutes after irradiation each individual sample, as well as the calcium carbonate standard, was transferred from its irradiation container into an unirradiated polyethylene vial and transferred quickly to a sodium-iodine multi-channel gamma-ray spectrometer for collection of a gamma-ray spectrum of the induced radioactivity within each sample and standard. The necessity for quick handling of the samples and standard and collection of data is due to the fact that the radioactive isotope calcium-49, which is produced when calcium is irradiated and whose activity was used to determine the amount of calcium present in the samples, decays with a half-life of 8.8 min.

Phosphorus. Twelve days after irradiation, the stapes samples and the cortical bone samples were mounted on aluminum discs using thin mylar tape and their beta particle activity analyzed using a beta sensitive 2 π proportional counter.

Initially, aluminum absorbers were used to determine the maximum beta particle energy present in the samples as well as any necessary background correction (Overmann & Clark, 1960). Also, the beta activity present in the samples was recorded every other day for fourteen days to determine the half-life of the beta-emitting isotope present in the sample. Using these techniques, it was determined that the maximum beta particle energy was 1.7 Mev and that the half-life was 14.3 days. Both of these values are characteristic of the radioactive isotope phosphorus-32, which is produced when natural phosphorus is irradiated. The amount of phosphorus in each of the samples was then determined using the beta activity recorded during this analysis.

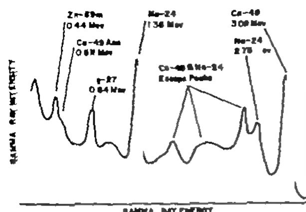


Fig 1 Typical gamma-ray spectrum of a stapes.

RESULTS

A typical example of a gamma-ray spectrum collected from the induced radioactivity of the samples analyzed during this study is shown in Fig. 1 Gamma rays from radioactive isotopes of zinc, magnesium, sodium, as well as calcium can be seen.

The calcium and phosphorus content in sixteen stapes samples, half being from otosclerotic and half being from non-otosclerotic patients, show no significant differences (Table 1) Likewise, no significant difference is seen when comparing cortical bone from otosclerotic individuals to cortical bone from non-osclerotic individuals.

DISCUSSION

Several investigators (Fowler 1948 Seiferth, 1937 Breytmann, 1933 Riskaer 1949) have attempted to determine if the calcium and phosphorus concentrations in serum from individuals with otosclerosis differ from the concentrations in serum from individuals without otosclerosis. The results have been conflicting. Calcium and phosphorus concentrations in osseous tissues from individuals with otosclerosis have been investigated by Maurer (1961/1962 and 1967) In his investigation, calcium was determined by permanganate after oxalate precipitation, and phosphorus was determined colorimetrically by amidol and ammonium molybdate Maurer's results indicated that the concentrations of calcium and phosphorus were lower in ossicles from individuals with otosclerosis.

With the use of the ultra-sensitive technique of neutron activation analysis, we have been able to determine calcium and phosphorus concentrations in osseous tissues which are in the same range as those reported by Maurer (1961/1962 and 1967) We were not able, however to demonstrate that the calcium and phosphorus concentrations in osseous tissue from otosclerotics differed significantly from those in osseous tissue from individuals without otosclerosis. Analyzed statistically cal-

Table 1 *Calcium and phosphorus concentration in stapes and cortical bone determined by neutron activation analysis*

Sample	Weight (mg)	mg Calcium/ mg bone	mg Phosphorus/ mg bone
(1) Control stapes (8)	2.778	0.328	0.113
	3.452	0.297	0.111
	2.601	0.297	0.109
	2.508	0.305	0.113
	2.202	0.301	0.116
	2.159	0.281	0.108
	2.091	0.264	0.118
	3.390	0.265	0.102
		0.292 ±0.008[a]	0.111 ±0.002
(2) Otosclerotic stapes (8)	2.561	0.295	0.107
	4.916	0.257	0.099
	2.203	0.315	0.107
	2.686	0.286	0.113
	2.202	0.300	0.132
	2.770	0.277	0.102
	2.541	0.278	0.118
	3.948	0.266	0.105
		0.284 ±0.007	0.110 ±0.004
(3) Control cortical bone (2)	2.655	0.247	0.076
	1.329	0.268	0.088
		0.258 ±0.011	0.082 ±0.006
(4) Cortical from otosclerotics (5)	3.157	0.255	0.093
	1.284	0.369	0.086
	3.930	0.323	0.114
	3.427	0.303	0.098
	4.639	0.263	0.088
		0.303 ±0.021	0.096 ±0.005

[a] Standard error of mean.

cium and phosphorus concentrations in normal stapes did not differ significantly from those in otosclerotic stapes or in cortical bone from otosclerotics.

Also the calcium to phosphorus ratios in the various osseous tissues examined did not differ It would be expected that the results obtained with a sensitive procedure such as neutron activation analysis would be consistent with the difference reported by Maurer if such a difference does exist in otosclerosis. The inconstancy between these two reports remains unexplained.

Attempts to correlate calcium and phosphorus concentrations in the tissue investigated with respect to age, sex, or degree of otosclerotic growth in footplates also were negative. The calcium and phosphorus concentrations on the basis of a dry weight of bone show no difference in either lamellar or non-lamellar bone.

The original intent of this investigation was to use the levels of calcium and phosphorus as in situ controls for further investigation of the additional elements in the osseous tissues from individuals with otosclerosis. The quantitative analysis of the elements other than calcium and phosphorus will be reported in a subsequent paper

ACKNOWLEDGMENT

The authors wish to give acknowledgment and thanks to: Colonel Philip S. Gwynn, Director of the AF NEC, for his enthusiastic encouragement, suggestion of analytical procedures and continuous strong support for continuation of this effort. M *Clyde Hill*, Systems Research Laboratories, Inc., Dayton, Ohio; TSgt Robert L. Schwig and Sgt. Francis W N ... of the AF NBC, for their help in performing the activation analysis discussed in this study

We wish to express our appreciation to Mrs. Sarah Thayer for her assistance in preparing this manuscript.

Special acknowledgment and thanks to the Department of Pathology Good Samaritan Hospital, Dayton, Ohio, for their cooperation in obtaining normal ossicles.

ZUSAMMENFASSUNG

Die Neutronen-Aktivationsanalyse wurde benutzt, um die anorganischen Elemente im Steigbügel und Knochenteilchen vom proximalen Ende der hinteren Gehörgangswand zu bestimmen. Zink, Natrium, Magnesium, Kalzium und Phosphor wurden entdeckt. Die Kalzium- und Phosphorkonzentrationen im Steigbügel und Knochenteilchen vom proximalen Ende der hinteren Gehörgangswand von Patienten mit Otosklerose wurden mit den Knochen von otosklerosefreien Kontrollen verglichen. Kein bedeutsamer Unterschied wurde festgestellt.

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EMBRYONAL RHABDOMYOSARCOMA OF THE MIDDLE EAR PRIMARY TO SO-CALLED MEDULLOMYOBLASTOMA OF BRAIN

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Received March 22, 1969

Two cases in children of embryonal rhabdomyosarcoma of the middle ear is presented with clinical, radiologic and microscopic findings. They had the same characteristics as the 20 previously described cases and early diagnosis was difficult, causing late treatment. Despite intens irradiation, none of the patients survived 7 months and the prognosis is considered very poor

In infants and children the rhabdomyosarcoma is the most common of the soft tissue sarcomas, frequent sites being the head, neck and the pelvis (Soule *et al.*, 1968) Within the head-neck region the middle ear is a rare localization, only some 20 cases being on record (Potter 1966 Kissane & Smith, 1967 Marsden & Stewart, 1968 Neidhardt, 1968, Nelson, 1968). Despite its rarity rhabdomyosarcoma of the middle ear warrants report for two reasons. First, it is difficult to diagnose both clinically and radiologically since it might, for a long time, mimic a chronic otitis owing to the presenting signs of polypoid (botryoid) granulations of the outer auditory canal. Second, as will be evident from this paper the middle ear rhabdomyosarcoma is probably the origin of some cases of so-called medullomyoblastoma of the cerebellum (Zülch, 1956 Russel & Rubinstein, 1959 Shuangshoti *et al* 1968). Two cases of embryonal rhabdomyosarcoma of the middle ear both with intracranial spread, will be presented to illustrate these points.

CLINICAL HISTORIES

Case 1

This was a five year-old girl who was admitted to hospital in April 1964 after a few days of illness with headache and vomiting as predominant symptoms. Soon she developed a nasal speech and difficulties in swallowing. A paralysis of the soft palate and of the left side of the pharynx was registered and an encephalitis was suspected until the girl after about 8 weeks of illness indicated ache in her left ear The ear drum was red and bulging and her symptoms seemed to derive from an acute otitis in the left ear An X ray of the skull was considered normal (see radiology) Antibiotic treatment was given without improvement and in the early May of 1964 a grayish-red growth was seen in the external auditory canal. The growth was considered to be a boil in the bottom of the canal. A biopsy was taken (see Table 1) Very soon thereafter a left-sided facial palsy developed. A new X ray of the temporal bone was taken and showed destructions in the middle ear As these were considered to be caused by osteomyelitis, a mastoidectomy was done on the 15th of May In the antrum and in the tympanic cavity the surgeon found granulomatous tissue reminiscent of tumor rather than usual infectious granulations. They also bled very easily A new biopsy was taken (Table 1) The girl's general condition



Fig 3 Case 2. Tomogram, Stenvers projection of the same level in the pyramids. On the right side (H) normal picture. On the left a destruction of the entire pyramidal apex to the cochlea, the walls of which are partially destroyed.

cellular systems of both middle ears were poorly developed and the left side showed decreased aeration. No definite destructions were seen. Three weeks later encephalography revealed the left cerebello-pontine cistern to be elevated and deformed laterally with a suspect filling of the cistern. This region corresponded to the upper margin of the left pyramid above the internal porus. Tomography of the middle cerebral fossa displayed a destruction of the medial part of the left pyramid. On June 18 a tomography of the ear showed a large destruction in the region of the left middle ear inner part of the outer auditory canal and of parts of the apex of the pyramid (Figs. 2 and 3) No auditory ossicles were seen on the left side and large portions of the pyramidal portion of the carotid canal were destroyed The jugular fossa was preserved The localization and appearance of the lesions made a neoplasm very likely Repeated examinations on June 24 and 29 showed rapid progress of the destructions.

PATHOLOGY

Case 1

Biopsies Four biopsies were taken during the course of the disease (May–August, 1964) They were all examined by experienced pathologists at a large center and the diagnoses varied from granulation tissue to neuroblastoma (Table I) All specimens displayed a polypoid tissue with mucoid stroma and loosely arranged, round, polygonal or spindle-shaped cells with small, dark nuclei. No cross-striations were seen. The stroma was vascular (Fig. 4)

Autopsy The relevant findings were confined to the skull and brain. There were no distant metastases. The vault of the skull was normal. The meninges of the cerebellum and pons were gray opaque and thickened into a sarcomalike tumor in the left cerebello-pontine angle measuring about 2 × 1 × 1 cm (Fig. 5). The left petrous bone was spongy and soft. On chiseling, a grayish opaque tissue was found to occupy most of its interior and seemed to be continuous with the meningeal process through the inner porus and continued along the auditory nerve

While the petrous bone was being decalcified, sections from the cerebello-pontine region was examined. They contained a pleomorphic tumor tissue, in part with the same features as were present in the biopsies. In addition there were several areas of long spindle-shaped cells, of which many displayed cross-striations (Fig. 6) At this time the tumor was thought consistent with the medullomyoblastoma of Zülch (1956)

The decalcified tissue from the petrous bone was composed of all previously described tu-



Fig. 4 Case 1. Biopsy from granulations of the left outer ear canal. Note vascular mucoid stroma and small cells with dark nuclei. N rhabdomyoblasts or cross-striated cells are present in the tissue. Hematoxylin-eosin. 190.

mor components. The middle ear was completely filled with tumor consisting of small, round or spindle-shaped cells in a mucoid stroma and, in addition, large, round cells with large vesicular nuclei, thought to be rhabdomyoblasts (Fig. 7). Long spindle-shaped cells with cross striations were also present in a moderate number

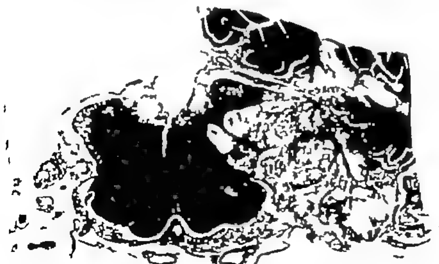


Fig. 5 Case 1 Meningeal growth of tumor in left [c]erebello-pontine angle, blocking the foramina of the 4 ventricle, but with no growth into the ventricular system. Before the decalcified left petrous bone was available for study this cerebral growth was interpreted as medullomyoblastoma owing to the presence of the cross-striated cells (see text and Fig. 6). PTAH. 3



Fig. 3. Case 2. Tomogram. Stenver's projection of the same level in the pyramids. On the right side (*H*) normal picture. On the left a destruction of the entire pyramidal apex to the cochlea, the walls of which are partially destroyed.

cellular systems of both middle ears were poorly developed and the left side showed decreased aeration. No definite destructions were seen. Three weeks later, encephalography revealed the left cerebello-pontine cistern to be elevated and deformed laterally with a suspect filling of the cistern. This region corresponded to the upper margin of the left pyramid above the internal porus. Tomography of the middle cerebral fossa displayed a destruction of the medial part of the left pyramid. On June 18 tomography of the ear showed a large destruction in the region of the left middle ear, inner part of the outer auditory canal and all parts of the apex of the pyramid (Figs. 2 and 3). No auditory ossicles were seen on the left side and large portions of the pyramidal portion of the carotid canal were destroyed. The jugular fossa was preserved. The localization and appearance of the lesions made a neoplasm very likely. Repeated examinations on June 24 and 29 showed rapid progress of the destructions.

PATHOLOGY

Case I

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The decalcified tissue from the petrous bone was composed of all previously described



Fig. 4 Case 1 Biopsy from granulations of the left outer ear canal. Note vascular mucoid stroma and small cells with dark nuclei. No rhabdomyoblasts or cross-striated cells are present in the tissue. Hematoxylin-eosin. × 190.

mor components. The middle ear was completely filled with tumor consisting of small, round or spindle-shaped cells in a mucoid stroma and, in addition, large, round cells with large vesicular nuclei, thought to be rhabdomyoblasts (Fig. 7) Long spindle-shaped cells with cross striations were also present in a moderate number



g. 5 Case 1. Meningeal growth of tumor in left rebello-pontine angle, blocking the foramina of the ventricle, but with no growth into the ventric y system. Before the decalcified left petrous was available for study this cerebral growth was interpreted as medullomyoblastoma owing to resence of the cross-striated cells (see te 6). PTAH. 3



Fig 6 Case 1 Large magnification from tumorous area depicted in Fig. 5 Spindle-shaped cells with cross-striated cytoplasm are clearly seen. PTAH 480

After the decalcification of the petrous bone the concept of the origin of the tumor was changed and the final diagnosis of embryonal rhabdomyosarcoma of the middle ear with meningeal metastases was established. This was 7 months after the first biopsy

Case 2

Biopsies Three biopsies were performed. The first two (June 5 and 14) were interpreted by an experienced pathologist as a malignant tumor probably originating in the ganglion cocum ("paraganglioma") The third, done a

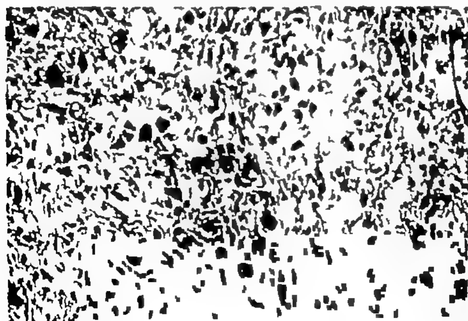


Fig 7 Case 1 Left petrous bone Large cells of rhabdomyoblastic type are present in the center Also, small polygonal cells, predominating in the ear canal, are interspersed with long, slender spindle-shaped cells. Some of these show cross striation at power (not visible). PTAH. 190.



Fig. 8 Case 2. Biopsy from outer ear canal. Poorly differentiated embryonal sarcoma, essentially similar to the biopsies from Case 1 (Fig. 4). Hematoxylin-eosin. 190

another hospital was diagnosed as embryonal rhabdomyosarcoma of the middle ear (June 28) The histologic details were the same as found in the polypoid granulations of Case 1 (Fig. 8) No rhabdomyoblasts of cross-striated cells were demonstrated, not even in PTAH stained tissue. A contributing factor facilitating the final diagnosis was the pathologist's familiarity with Case 1

Autopsy The *post mortem* findings were very similar to those of Case 1 The left petrous bone was very soft and spongy The infratentorial meninges were opaque and—in view of the findings in Case 1—this change was sought for and found also on the surface of the left auditory nerve In contrast to Case 1 however there was no circumscribed tumor in the brain. The brain weighed 1468 grams expected weight 1200 g) and showed edema. The microscopic sections revealed diffuse sarcomatosis of the infratentorial meninges. As in Case 1 there were numerous areas with elongated cells displaying cross striations. The microscopic picture of the decalcified left petrous bone was essentially similar to that of Case 1 including the presence of cross-striated cells.

The stomach and duodenum contained a few small punched-out ulcers of Cushing-type Otherwise the viscera were normal

DISCUSSION

The two patients with embryonal rhabdomyosarcomas of the middle ear presented show an almost identical picture. They are also in full agreement with cases reported earlier (Maconie, 1944 Holman, 1956 Mehra, 1960· Vidal, 1964)

The described cases have all been children who have presented themselves with easily bleeding granulations in the external auditory canal and with early neurological symptoms. On X-ray there have been extensive, rapidly progressive destructions in the petrous and mastoid temporal bones. In almost all of the reported cases the changes in the middle ear have been regarded as inflammatory in origin and they have been subjected to mastoid operations. As this operation in some cases to have stimulated the tumor-growth (Mr 1944 Vidal, 1964) the clinician sho

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MUCOVISCIDOSIS AND NASAL POLYPS

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A case of MV with pronounced nasal symptoms in a 12 year-old girl is described. It is important that the patient should come under treatment as early as possible because of the long-term prognosis, and here the otologist can make his contribution. Since the completion of this paper another patient with suspected mucoviscidosis has been examined at the clinic. This is an 8-year-old girl who was referred to the clinic because of recurring nasal polyps and otitis. A polyp was found in the left nostril and there was opacity of the maxillary sinuses. The amount of sodium in sweat, determined by pilocarpine iontophoresis a.m. Gibbson & Cooke, was increased (81 and 86 mEq/l) and there was an elevated sodium/chlor ratio (5.4 and 6.1). The patient has been ad to the Paediatric Department of Blegdams for further examination for mucoviscidosis.

Mucoviscidosis (MV) previously designated fibrosis of the pancreas or cystic fibrosis, often produces symptoms that can be observed by the otologist. Thus the otologist can contribute to the early diagnosis and treatment, and perhaps to a prolongation of the patient's life

The underlying cause of the disease is a defect in the muciparous glands, which causes the secretion to be more viscous and results in the obstruction of the excretive ducts. The sweat, salivary and lacrimal glands may also be [illegible]tacked. Investigation of secretion has revealed abnormal mucoprotein with a high content of fucose and sialic acid, and sweat examination has shown that *the sweat from these patients* contains considerably higher concentrations of sodium and chloride than those normally found.

The symptomatology varies considerably dependent on which of the organs are the most affected It should be mentioned here, on account of the original name of the disease, that the pancreas need not necessarily be involved. Ten per cent of the patients die at birth from meconium ileus. Partial or total obstruction of the bronchi leads to emphysema and atelectasis, recurrent pneumonia and finally pulmonary fibrosis with cyanosis and clubbing of the fingers. The intestinal symptoms resemble coeliac syndrome There may be hepatic cirrhosis, gastric ulcers and rectal prolapse

Of special interest for the otologist are the early symptoms from the nose and sinuses, such as recurrent sinusitis, nasal stenosis and nasal polyps. The finding of a patient with MV cannot be a rare occurrence, since American writers report that this recessive hereditary disease is demonstrated in one out of every thousand live births in America, and that 10% of the patients have nasal polyps. According to Schwachman *et al* (1962) these are observed as early as at the age of 2 years. X-ray examination of the sinuses shows dense opacity of the maxillary and possibly other sinuses. Puncture of the maxillary sinuses generally reveals a thick greyish-green mucus Microscopy *of the sinus mucous membrane or nasal polyps* demonstrates large, distended submucous glands in an oedematous membrane The nasal polyps may be very large and may cause such



Fig 1



Fig 2

pressure on the nasal structure that saddle nose and broadened nasal bridge may result if the patient lives for many years (Fig. 1)

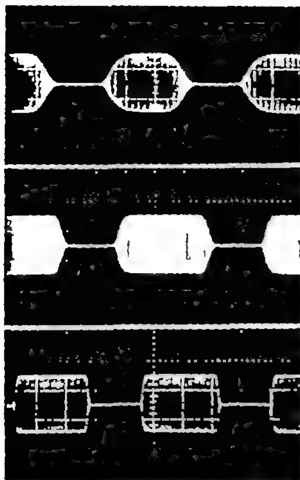
Allergy has been suggested as a contributory factor in nasal polyps, but there is no definite proof of this. However antihistamine and antiserotonine treatment seems to have some effect on the growth of the polyps.

The increased content of sodium and chloride in the sweat of MV patients can be utilized in diagnosis. Gahn & Schwachman published in 1956 a simple method for determination of that condition. The patient s hand is placed on an agar plate prepared with silver nitrate and potassium chloride. If the patient s sweat is normal, only a weak precipitation corresponding to the hand-print will occur on the plate, whereas in the case of patients with MV the precipitation is considerable. The sweat can also be examined by means of pilocarpine iontophoresis. Furthermore, the diagnosis can be made on the basis of roentgenogram of the lungs, revealing pulmonary fibrosis and atelectasis, and X-ray examination of the paranasal sinus, enzyme investigation (particularly of duodenal secretion), liver function tests and stool examination. Previously the prognosis was very poor and the average length of life after establishment of the diagnosis was [illegible] years In some cases, however the diagnosis is made in older children or adults (the less severe cases) often because of recurrent sinusitis and nasal polyps. Early diagnosis and treatment are of course of great significance, and here the otologist can contribute by bearing in mind the possibility of mucoviscidosis when observing recurrent nasal polyps in children and young persons.

The usual treatment consists of the prevention of pneumonia and atelectasis by means of respiration exercises, the use of ultrasonic



Fig 3



1. Photographic record of the oscillographic pic of the three tone pulses. *Upper* pulse length is msec with a rise and decay time of 50 msec ˜d an off time of 170 msec. *Centre* (pulse length 500 msec), the rise and decay time is 60 msec and the off period 300 msec. *Lower* the pulse length of 1300 msec has a rise and decay time of 50 msec with an off time of 900 msec. The scale is different in each oscillogram.

pulse as well as the on and off times were recorded. The pulse durations thus obtained for the rate of /₂ pulse/sec were 1300 msec with an off time of 900 msec, the rise and decay times being 50 msec The corresponding values for the pulse frequency of 1 pulse/sec were 500 300 and 60 msec, and for 2 pulses/sec, 170 170 and 50 msec, respectively (Fig. 1) The rate of intensity change was 2.5 and 5 dB/sec The studies were started from 250 Hz proceeding to the higher tones up to 8000 Hz In addition to the fulloctaves, the tones of 1500 3000 5000 6000 and 7000 Hz were included. The time used for threshold determination at each frequency was 30 secs, and thus testing all frequencies took about 5 min, while the testing time, employing all combinations, was about 40 min for one ear The method of adjustment was used, the subjects pressing the key as long as the tone was heard and releasing it as soon as it disappeared

From the threshold excursions, the maximal and minimal values were tabulated and the midpoint of these two figures was recorded as the threshold for that particular frequency The statistical treatment of the data took place at the computer centre of the University of Oulu. Student's *t* test was used in determination of the statistical significancies, and the value $p \leq 0.01$ was taken as the level for significant differences.

RESULTS

Fig. 2 shows the average threshold curves obtained with the three different pulse durations, 170 500 and 1300 msec, for the various frequencies. The interrupted line marks the thresholds obtained with the rate of 2.5 dB/sec and the continuous line those obtained with the rate of 5 dB/sec At low frequencies, the curves show an even level at about 10–15 dB (ISO) and then a gradual decline from 1000 Hz to about 55 dB for 5000–8000 Hz. The range between minimal and maximal average thresholds is 15–20 dB for all frequencies.

To study the effect of pulse length on the thresholds, the individual differences were tabulated for each pair of pulse duration under comparison and the average differences calculated. These are shown in Table I with the standard deviations for various pulse length comparisons. Application of *t*-test to these data showed that there were no significant differences between the thresholds obtained with the three pulse durations at any frequency concerned ($p > 0.05$) This overlapping of data is similar for both rates of intensity change. 2.5 or 5 dB/sec.

Comparison of the data obtained with a con-

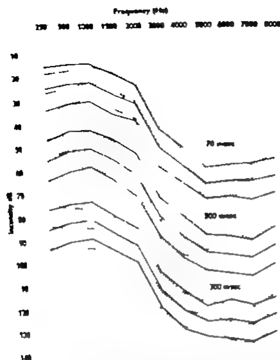


Fig 2 Average thresholds (ISO-standard) in 41 ears ith presbyacusis as function f pulse length and intensity change. The two lower groups with 500 and 1700 msec pulse length are artificially separated from the 170 msec group by 30 and 60 dB respec tively The middle curves in each group show the average thresholds while the other two curves show the minimal and maximal average thresholds. An intensity change of 2.5 dB/sec is indicated by broken line, 5 dB/sec by a continuous line.

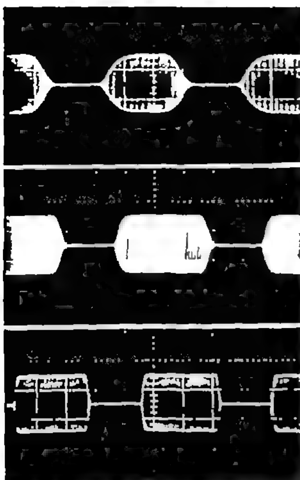
stant pulse length and with an intensity change of 2.5 or 5 dB/sec (Table 2) revealed no statistically significant effects on the thresholds ($p > 0.05$ in all combinations) The thresholds tended to be slightly better with the short pulses at the rate of 2.5 dB/sec than with longer pulses. On the other pulses of 1300 msec gave slightly better thresholds with an intensity change of 5 dB/sec.

Table 3 shows the effect of the two rates of intensity change and the three pulse tone durations on the threshold amplitudes at various frequencies. With the rate of 2.5 dB/sec, the excursion amplitudes were slightly larger for 1300 msec tones than for 170 or 500 msec tones at all frequencies. The differences were significant at the level of 0.05 for 4000 Hz between 170 and 500 msec tone pulses, between 170 and 1300 msec pulses for 500, 1000 and 6000 Hz, and between 500 and 1300 msec for 500 and 7000 Hz. Using the rate 5 dB/sec the differences were clearly less marked Statistically the differences were only suggestive between the tones of 170 and 1300 msecs ($p < 0.05$) at 2000 Hz and significant at 4000 Hz ($p < 0.01$)

With the 170 msec pulses the excursions were generally smaller for 2.5/sec intensity change than for 5 dB/sec. The differences were significant ($p < 0.01$) at 500 Hz and suggestive at 1500 and 4000 Hz ($p < 0.05$) With a pulse length of 500 msec, the excursion amplitudes were also smaller at the slow rate (2.5 dB/sec) of intensity change than at the fast rate (5 dB/sec) Statistically the differences were significant ($p < 0.01$) for 2000 Hz and suggestive ($p < 0.05$) for 500 5000 7000 and 8000 Hz. As for the tone pulses of 1300 msec duration, the rate of 2.5 dB/sec sometimes produced even larger threshold amplitudes than obtained by the rate 5 dB/sec but, statistically none of the values reached the significance level of 0.05

DISCUSSION

In normally hearing young adults, tones exceeding 150 msec provide loudness equal to longer tones (Garner 1948) and thus identical thresholds. Tones shorter than 150 msec are likely to give poorer thresholds than longer tones even if the difference becomes critical only for tones shorter than 100 msec (Garner 1949) In recent studies comparison of continuous tones with 130 250 and 500 msec pulse tones showed identical thresholds (Palva, 1956) Similar results were reported by Plomp & Bouman (1959) Caspers *et al* (1960) and Taniewski (1967) though, according to the latter two, tones shorter than 500 msec might already give poorer thresholds than longer tones In all these comparisons the requirement was that the silent interval is sufficiently long if it is kept very short, short duration



1 Photographic record of the oscillographic pic of the three tone pulses. *Upper* pulse length is 1 msec with a rise and decay time of 50 msec d an off time of 170 msec *Centre* (pulse length 500 msec), the rise and decay time is 60 msec and the off period 300 msec. *Lower* the pulse length of 1300 msec has a rise and decay time of 50 msec with an off time of 900 msec. The scale is different in each oscillogram.

pulse as well as the on and off times were recorded. The pulse durations thus obtained for the rate of $^1/_2$ pulse/sec were 1300 msec with an off time of 900 msec, the rise and decay times being 50 msec The corresponding values for the pulse frequency of 1 pulse/sec were 500 300 and 60 msec, and for 2 pulses/sec, 170, 170 and 50 msec, respectively (Fig. 1) The rate of intensity change was 2.5 and 5 dB/sec. The studies were started from 250 Hz proceeding to the higher tones up to 8000 Hz, in addition to the fulloctaves, the tones of 1500 3000 5000 6000 and 7000 Hz were included. The time used for threshold determination at each frequency was 30 secs, and thus testing all frequencies took about 6 min, while the testing time employing all combinations, was about 40 min for one ear The method of adjustment was used, the subjects pressing the key as long as the tone was heard and releasing it as soon as it disappeared

From the threshold excursions, the maximal and minimal values were tabulated and the midpoint of these two figures was recorded as the threshold for that particular frequency The statistical treatment of the data took place at the computer centre of the University of Oulu. Student's *t*-test was used in determination of the statistical significances, and the value $p \leq 0.01$ was taken as the level for significant differences.

RESULTS

Fig. 2 shows the average threshold curves obtained with the three different pulse durations, 170 500 and 1300 msec, for the various frequencies. The interrupted line marks the thresholds obtained with the rate of 2.5 dB/sec and the continuous line those obtained with the rate of 5 dB/sec At low frequencies, the curves show an even level at about 10–15 dB (ISO) and then a gradual decline from 1000 Hz to about 55 dB for 5000–8000 Hz. The range between minimal and maximal average thresholds is 15–20 dB for all frequencies.

To study the effect of pulse length on the thresholds, the individual differences were tabulated for each pair of pulse duration under comparison and the average differences calculated. These are shown in Table 1 with the standard deviations for various pulse length comparisons Application of *t*-test to these data showed that there were no significant differences between the thresholds obtained with the three pulse durations at any frequency concerned ($p > 0.05$) This overlapping of data is similar for both rates of intensity change, 2.5 or 5 dB/sec.

Comparison of the data obtained with a con-

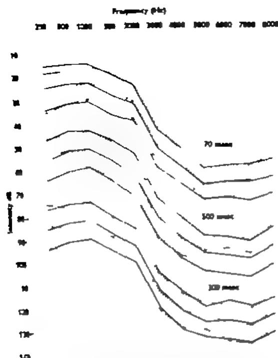


Fig 2 Average thresholds (ISO-standard) in 41 ears with presbyacusis as a function f pulse length and intensity change. The two lower groups with 500 and 1700 msec pulse length are artificially separated from the 170 msec group by 30 and 60 dB, respec tively The middle curves in each group show the average thresholds while the other two curves show the minimal and maximal average thresholds. An intensity change f 2 5 dB/sec is indicated by broken line, 5 dB/sec by a continuous line.

stant pulse length and with an intensity change of 2.5 or 5 dB/sec (Table 2) revealed no sta tistically significant effects on the thresholds ($p > 0\ 05$ in all combinations) The thresholds tended to be slightly better with the short pulses at the rate of 2.5 dB/sec than with longer pulses. On the other pulses of 1300 msec gave slightly better thresholds with an intensity change of 5 dB/sec.

Table 3 shows the effect of the two rates of intensity change and the three pulse tone durations on the threshold amplitudes at various frequencies. With the rate of 2.5 dB/sec, the excursion amplitudes were slightly larger for 1300 msec tones than for 170 or 500 msec tones at all frequencies. The differences were significant at the level of 0 05 for 4000 Hz between 170 and 500 msec tone pulses, be tween 170 and 1300 msec pulses for 500 1000 and 6000 Hz, and between 500 and 1300 msec for 500 and 7000 Hz. Using the rate 5 dB/sec the differences were clearly less marked. Statistically the differences were only suggestive between the tones of 170 and 1300 msecs ($p < 0\ 05$) at 2000 Hz and significant at 4000 Hz ($p < 0\ 01$)

With the 170 msec pulses the excursions were generally smaller for 2.5/sec intensity change than for 5 dB/sec. The differences were significant ($p < 0\ 01$) at 500 Hz and suggestive at 1500 and 4000 Hz ($p < 0\ 05$) With a pulse length of 500 msec, the excursion amplitudes were also smaller at the slow rate (2.5 dB/sec) of intensity change than at the fast rate (5 dB/sec) Statistically the differences were significant ($p < 0.01$) for 2000 Hz and suggestive ($p < 0\ 05$) for 500 5000 7000 and 8000 Hz. As for the tone pulses of 1300 msec duration, the rate of 2.5 dB/sec sometimes produced even larger threshold amplitudes than obtained by the rate 5 dB/sec but, statistically none of the values reached the significance level of 0 05

DISCUSSION

In normally hearing young adults, tones ex ceeding 150 msec provide loudness equal to longer tones (Garner 1948) and thus identical thresholds Tones shorter than 150 msec are likely to give poorer thresholds than longer tones even if the difference becomes critical only for tones shorter than 100 msec (Garner 1949) In recent studies comparison of continuous tones with 130 250 and 500 msec pulse tones showed identical thresholds (Palva, 1956) Similar results were reported by Plomp & Bouman (1959) Caspers *et al* (1960) and Taniowski (1967) though, according to the lat ter two tones shorter than 500 msec might already give poorer thresholds than longer tones. In all these comparisons the require ment was that the silent interval is sufficiently long: if it is kept very short, short duration

Table 1 *Average differences in thresholds obtained by various pulse durations and rates of intensity change*

A negative sign indicates that the threshold was poorer with the longer pulse duration

Pulse duration (frequency)	170–500 msec		170–1300 msec		500–1300 msec	
	Average difference	s.d.	Average difference	s.d.	Average difference	s.d.
Intensity change 2.5 dB/sec						
250	2.6	8.8	2.6	8.9	−0.1	8.7
500	3.0	6.6	0.1	8.8	−2.9	9.2
1000	1.6	5.0	−2.8	8.3	−4.4	7.1
1500	0.7	5.4	−2.6	9.0	−3.3	7.9
2000	−1.1	5.7	−5.1	[illegible]	−4.0	7.1
3000	−2.8	4.4	−3.6	8.4	−0.8	6.7
4000	−2.2	5.8	−3.6	9.2	−1.5	6.6
5000	1.7	6.6	3.0	13.8	1.4	18.2
6000	[illegible]	5.2	−0.7	8.1	−2.4	7.8
7000	1.1	12.2	−2.6	8.6	−1.5	11.4
8000	0.0	7.3	−0.7	8.2	−0.8	9.1
Intensity change 5 dB/sec						
250	0.9	8.9	−0.7	6.7	0.2	7.4
500	1.0	6.6	0.3	6.9	−0.7	4.5
1000	1.7	4.8	1.2	6.1	−0.5	7.3
1500	2.0	5.3	0.2	8.4	−1.8	7.4
2000	0.4	6.1	−0.9	8.5	−0.5	7.0
3000	0.5	7.0	−0.9	6.4	−1.4	5.8
4000	−0.5	5.7	−2.2	6.0	−1.7	5.2
5000	0.2	4.1	0.5	6.2	0.3	7.6
6000	−1.7	9.6	0.0	9.8	1.7	4.9
7000	−3.1	6.4	−1.5	6.4	1.6	6.5
8000	0.3	6.8	0.4	8.9	0.1	11.0

Table 2. *Average differences in thresholds obtained by 2.5 and 5 dB/sec intensity change at various pulse durations*

A negative sign indicates that the threshold obtained by the 2.5 dB/sec rate was poorer than the 5 dB/sec rate threshold

Pulse duration (frequency)	170 msec		500 msec		1300 msec	
	Average difference	s.d.	Average difference	s.d.	Average difference	s.d.
250	−2.0	6.6	1.5	[illegible]	1.2	8.7
500	−1.7	7.3	0.3	7.4	−1.9	8.9
1000	0.1	3.9	0.1	7.5	−3.8	10.7
1500	1.4	6.3	0.1	7.1	−1.4	10.1
2000	0.7	6.2	0.0	7.4	3.5	10.2
3000	1.5	5.7	−1.8	[illegible]	−1.2	8.1
4000	1.2	10.7	−0.4	4.0	0.2	8.0
5000	−0.5	6.7	0.9	7.3	2.0	21.7
6000	−0.4	10.4	3.1	6.6	1.0	11.5
7000	0.5	6.4	2.4	9.6	0.6	8.8
8000	2.5	10.7	2.2	10.6	1.3	11.6

Table 3. *Excursion amplitudes as a function of pulse duration and the rate of intensity change*

Pulse duration (frequency)	170 msec		500 msec		1300 msec	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
Intensity change 2.5 dB/sec						
250	17.0	7.2	15.4	5.4	19.3	11.8
500	14.3	4.1	14.2	[illegible]	17.2	7.2
1000	13.7	4.5	15.1	4.2	17.0	6.8
1500	14.8	4.6	15.3	4.1	17.3	[illegible]
2000	14.1	4.5	14.2	3.9	16.1	[illegible]
3000	14.3	5.1	14.5	5.3	16.5	6.1
4000	10.6	10.4	14.7	5.5	14.6	6.6
5000	12.0	5.6	8.4	13.6	11.2	17.0
6000	12.3	4.8	13.5	6.5	16.5	[illegible]
7000	13.4	6.2	10.0	11.5	[illegible]	7.2
8000	[illegible]	5.5	12.3	6.3	15.0	[illegible]
Intensity change 5 dB/sec						
250	18.7	7.5	17.3	6.0	17.2	7.7
500	17.0	8.7	16.9	4.4	16.7	[illegible]
1000	16.2	5.6	15.5	5.7	16.2	5.7
1500	17.3	4.4	17.2	5.0	15.1	[illegible]
2000	15.3	4.7	18.1	5.8	14.5	6.5
3000	16.0	5.8	16.2	6.4	15.6	7.1
4000	15.1	5.7	17.8	7.7	16.5	7.5
5000	13.1	10.1	15.1	11.2	13.2	12.6
6000	13.6	11.2	15.9	6.6	17.1	9.9
7000	15.6	7.2	15.5	6.4	16.2	8.0
8000	14.7	6.6	15.6	6.9	14.8	7.4

auditory fatigue may show its effect in threshold (de Maré, 1951) When the silent intervals exceed 100 msec, this phenomenon does not affect the threshold In Wittich's (1966) experience a 2000 Hz test tone of 250 msec at 53 dB SPL loses only 0 7 dB of its loudness level after a silent interval of 160 msecs.

In the present group of presbyacusis, 170, 500 or 1300 msec tone pulses, with silent intervals of 170 300 and 900 msec, respectively yielded similar hearing thresholds at all frequencies. This shows that a 170 msec tone has a sufficient length for full loudness value and that, with increasing length of the pulse tone to 1300 msec, the threshold remains similar to that of a short tone. Thus it seems to be the interruption procedure as such that causes the hearing thresholds in presbyacusis to be better than with continuous tone presentation (Jokinen, 1969)

A change in the intensity rate resulted, in a normal material (Corso, 1957) in about 5

dB better thresholds if the rate was rapid (5 dB/sec) than if it was slow (1.5 dB/sec). In this material, using the intensity rates 2.5 and 5 dB/sec, the thresholds were statistically the same for all different pulse rates.

The threshold amplitudes decreased as a function of frequency irrespective of the length of the pulsed tone. With the rate of 2.5 dB/sec intensity change, the threshold amplitudes were generally smaller for 170 and 500 msec tone pulses than for the same pulses at the rate of 5 dB/sec. Thresholds with 1300 msec tone pulses were similar at both intensity rates. Short tone pulses, in this study 170–500 msec, are likely to yield better hearing levels in presbyacusis than long pulses. Increased threshold amplitudes for 1300 msec tones are indicative of greater uncertainty around the threshold. Whether or not an adaptation has a part to play with these longer tone pulses, or continuous tones, is at present under study.

ZUSAMMENFASSUNG

Die Schwellenwerte wurden mit einem Rudmose ARJ 5 Audiometer bei 41 altersschwerhörigen Ohren bestimmt durch zwei Raten der Intensitätsveränderung (2,5 und 5 dB/sec) und bei Impulsdauern von 170, 500 und 1300 msec. Die stillen Intervalle betrugen jeweils 170, 300 und 900 msec. Die bei verschiedenen Kombinationen erhaltenen Schwellenwerte zeigten keine bedeutenden Differenzen bei der statistischen Auswertung. Der Umfang der Schwellenamplituden war geringer bei Tönen von 170 und 500 msec für mehrere Frequenzen als vergleichsweise bei Tönen von 1300 msec; unabhängig von den Testmethoden ergaben die niedrigen Töne grössere Amplituden als die hohen. Das beste Ergebnis wurde mit kurzen 170 msec Impulsen erhalten.

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Table 1 *Average differences in thresholds obtained by various pulse durations and rates of intensity change*

A negative sign indicates that the threshold was poorer with the longer pulse duration

Pulse duration (frequency)	170–500 msec Average difference	s.d.	170–1300 msec Average difference	s.d.	500–1300 msec Average difference	s.d.
Intensity change 2.5 dB sec						
250	2.6	8.8	2.6	8.9	−0.1	8.7
500	3.0	6.6	0.1	8.8	−2.9	9.2
1000	1.6	5.0	−2.8	8.3	−4.4	7.1
1500	0.7	5.4	−2.6	9.0	−3.3	7.9
2000	−1.1	5.7	−5.1	8.9	−4.0	7.1
3000	−2.8	4.4	−3.6	8.4	−0.8	6.7
4000	[illegible]	5.8	−3.6	9.2	−1.5	6.6
5000	1.7	6.6	3.0	13.8	1.4	18.2
6000	1.8	5.2	−0.7	8.1	−2.4	7.8
7000	−1.1	12.2	−2.6	8.6	−1.5	11.4
8000	0.0	7.3	−0.7	8.2	−0.8	9.1
Intensity change 5 dB/sec						
250	−0.9	8.9	−0.7	6.7	0.2	7.4
500	1.0	6.6	0.3	6.9	−0.7	4.5
1000	1.7	4.8	[illegible]	6.1	−0.5	7.3
1500	2.0	5.3	0.2	8.4	−1.8	7.4
2000	−0.4	6.1	−0.9	8.5	−0.5	7.0
3000	0.5	7.0	−0.9	6.4	−1.4	5.8
4000	−0.5	5.7	−2.2	6.0	−1.7	[illegible]
5000	0.2	4.1	0.5	6.2	0.3	7.6
6000	−1.7	9.6	0.0	9.8	1.7	4.9
7000	−3.1	6.4	−1.5	6.4	1.6	6.5
8000	0.3	6.8	0.4	8.9	0.1	11.0

Table 2. *Average differences in thresholds obtained at 2.5 and 5 dB/sec intensity change at various pulse durations*

A negative sign indicates that the threshold obtained by the 2.5 dB sec rate was poorer than the 5 dB/sec rate threshold

Pulse duration (frequency)	170 msec Average difference	s.d.	500 msec Average difference	s.d.	1300 msec Average difference	s.d.
250	−2.0	6.6	1.5	8.9	[illegible]	8.7
500	−1.7	7.3	0.3	7.4	1.9	8.9
1000	0.1	3.9	0.1	7.5	3.8	10.7
1500	1.4	6.3	0.1	7.1	1.4	10.1
2000	0.7	[illegible]	0.0	7.4	3.5	10.2
3000	1.5	5.7	−1.8	5.8	[illegible]	8.1
4000	1.2	10.7	0.4	4.0	−0.2	8.0
5000	−0.5	6.7	0.9	7.3	[illegible]	21.7
6000	−0.4	10.4	3.1	6.6	1.0	11.5
7000	0.5	6.4	2.4	9.6	0.6	8.8
8000	2.5	10.7	2.2	10.6	1.3	11.6

Table 3 *Excursion amplitudes as a function of pulse duration and the rate of intensity change*

Pulse duration (frequency)	170 msec Mean	s.d.	500 msec Mean	s.d.	1300 msec Mean	s.d.
Intensity change 2.5 dB sec						
250	17.0	7.2	15.4	5.4	19.3	11.8
500	14.3	4.1	14.2	5.2	17.2	7.2
1000	13.7	4.5	15.1	4.2	17.0	[illegible]
1500	14.8	4.6	15.3	4.1	17.3	8.1
2000	14.1	4.5	14.2	3.9	16.1	[illegible]
3000	14.3	5.1	14.5	5.3	16.5	[illegible]
4000	10.6	10.4	14.7	5.5	14.6	6.6
5000	12.0	5.6	8.4	13.6	[illegible]	17.0
6000	12.5	4.8	13.5	6.5	16.5	8.1
7000	13.4	6.2	10.0	11.5	15.2	7.2
8000	12.6	5.5	12.3	6.3	15.0	7.2
Intensity change 5 dB/sec						
250	18.7	7.5	17.3	6.0	17.2	7.7
500	17.0	8.7	16.9	4.4	16.7	[illegible]
1000	16.2	5.6	15.5	5.7	16.2	5.7
1500	17.3	4.4	17.2	5.0	15.1	[illegible]
2000	15.3	4.7	18.1	5.8	14.5	[illegible]
3000	16.0	5.8	16.2	6.4	15.6	7.1
4000	15.1	5.7	17.8	7.7	16.5	7.5
5000	13.1	10.1	15.1	11.2	13.2	[illegible]
6000	13.6	11.2	15.9	6.6	17.1	9.9
7000	15.6	7.2	15.5	6.4	16.2	[illegible]
8000	14.7	6.6	15.6	6.9	14.8	7.4

auditory fatigue may show its effect in threshold (de Maré 1951) When the silent intervals exceed 100 msec, this phenomenon does not affect the threshold. In Wittich's (1966) experience, a 2000 Hz test tone of 250 msec at 53 dB SPL loses only 0.7 dB of its loudness level after a silent interval of 160 msecs.

In the present group of presbyacusis, 170, 500 or 1300 msec tone pulses, with silent intervals of 170, 300 and 900 msec, respectively yielded similar hearing thresholds at all frequencies. This shows that a 170 msec tone has a sufficient length for full loudness value and that, with increasing length of the pulse tone to 1300 msec, the threshold remains similar to that of a short tone. Thus it seems to be the interruption procedure as such that causes the hearing thresholds in presbyacusis to be better than with continuous tone presentation (Jokinen, 1969)

A change in the intensity rate resulted, in a normal material (Corso 1957) in about 5



Fig. 1. Cochlear duct of the upper basal turn of normal si month old mink. Light micrograph f plastic-imbedded, toluidine blue stained 1.0 μ section. 245

F g. 2. Adult Hedlund mink pper basal turn. There is no endolymphatic space in the cochlear duct, and Reissner membrane is plastered against the stria vascularis, promontory outer sulcus cells and organ f Corti. The stria vascularis is atrophic. Out er hair cells are missing. Same technique and mag nification as F g. 1

lel to a plane tangent to the coil of the stria. In addition, three adult Hedlund mink and three normal adult mink were perfused with ferritin to study circulatory capability Commercially prepared ferritin (Mann Research Laboratories, N Y) was injected at a dose of 2.0 cc/K into the exposed left ventricle after ligation of the descending aorta. After three minutes, the animals were decapitated and the inner ears prepared for electron microscopy

Blood samples from normal and Hedlund adults were screened for arborvirus antibody by Jordi Casals, M.D., with his technique for hemagglutination-inhibition (Casals, 1961)

RESULTS

Figs. 1 and 2 are light microscopic comparisons of adult and Hedlund mink basal turn. The stria vascularis is obviously atrophic in the Hedlund specimen. The distance from the beginning of the stria epithelium above the spi ral prominence to its upper edge just beneath the takeoff of Reissner s membrane was de signated the width of the stria. The average width in the normal basal and middle turn measured approximately 250 μ In Hedlund mink, comparable specimens averaged a width



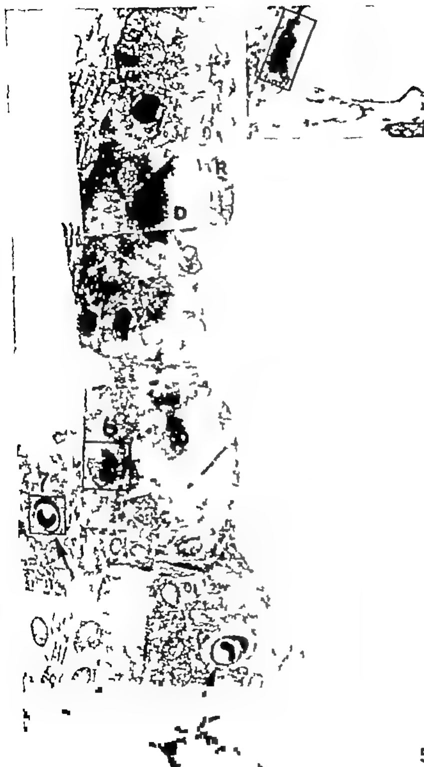
Fig 3 Normal mink stria vascularis and connective tissue of the spiral ligament. Connective tissue blood vessels (*C*) contain a lower concentration of erythrocytes than vessels of the epithelium of the stria (*S*). This is probably related to the normal slow rate of flow through strial vessels. Composite low magnification electron micrograph.



Fig 4 Ferritin particles in the blood plasma within a vessel of the stria vascularis. Between the erythrocyte (*RBC*) and the endothelial wall (*E*) a large number of fine opaque ferritin granules are seen. The endothelial cells are enveloping aggregates of granules by pinocytosis. This normal adult mink had been injected with ferritin three minutes before. ×45,000

of 140 μ. The distance from the endolymphatic surface to the deepest layer of the epithelium was called the thickness of the stria. It was measured at various points in a number of normal and Hedlunds. The thickness averaged 30 μ in normals and 18 μ in Hedlund mink.

Fig. 3 is a composite electron micrograph of normal mink stria vascularis. The vessels of the epithelium are filled with dark, homogene-



5

Fig. 5 Composite of eight low-magnification electron micrographs of the stria vascularis and spiral prominence of an adult Hedlund mink. The inset is light micrograph to depict area illustrated. Dark debris (*D*) collected between Reissner' membrane (*R*) and the outer surface f the stria vascularis. Vessels of the stria are filled with erythrocytes and clot. Connectiv tissue vessels are patent (*arrow*). Areas of Figs. 6 and 7 indicated.



Fig 6 Blood vessel of the stria vascularis in Hedlund adult which had been injected with ferritin. A platelet (*P*) seems attached to the endothelial wall, and membrane-bound debris from a degenerated leucocyte (*D*) occupies much of the lumen. No ferritin particles in the plasma, but cross cut fibrin strand (*arrows*) are found. 2..600.



Fig 7 Ferritin particle in plasma within vessel of connective tissue from beneath the stria vascularis in a Hedlund mink. A large number of very dense small particles of ferritin could be found in these vessels very soon after injection showing that their circulation was normal. 7,900.

ous material Most represents packed erythrocytes, but the plasma is also dark by comparison with that of a nearby vessel in the connective tissue beneath the epithelium. Fig. 4 is a higher power view of a normal vessel of the epithelium after ferritin injection. Small, dense particles of ferritin can be seen in the plasma space some are being engulfed by endothelial cells. This ferritin had been injected under physiological pressure only three minutes earlier

Fig. 5 is a very low magnification composite

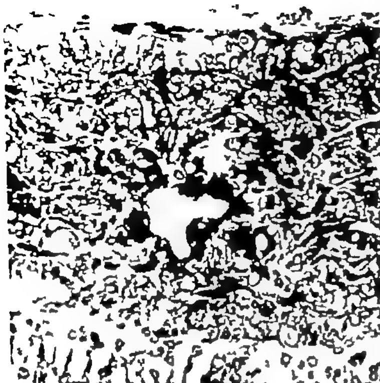


Fig 8 Normal stria vascularis sectioned tangent to cochlear spiral. There is a rich network of small blood vessels filled with erythrocytes. Many anastomotic branches are present. Toluidine blue stained section of plastic imbedded tissue light microscopy ×360.

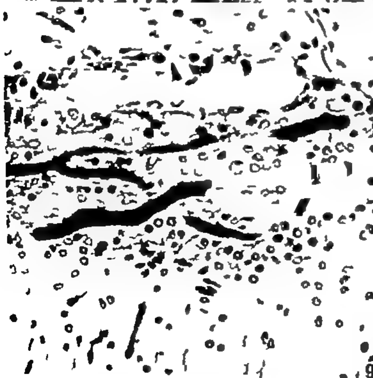


Fig 9 Hedlund stria vascularis processed identically. The vascular pattern is simpler and there are very few branches between the few remaining vessels. ×360.



Fig. 10. Low power electron micrograph of longitudinal section of Hedlund stria vessels. A degenerated leucocyte (*L*) and beginning aggregation of platelet (*P*). Fibrin clots with characteristic cross-striation were found (*circled area*). Showed at higher magnification in Fig. 11 × 74,000. Neighboring vessels permitted the passage of ferritin. × 5200.

electron micrography illustrating the same type of comparison between epithelial and connective tissue vessels in Hedlund mink. The lumen of the strial vessels is densely filled with homogeneous material. Fig. 6 shows a higher magnification view of an epithelial vessel after ferritin injection. Ferritin is absent, as was usually the case in Hedlund strial vessels, indicating poor circulation. Fig. 7 is a vessel from the connective tissue beneath the stria of the same Hedlund specimen. It contains a large amount of ferritin, showing that its perfusion was adequate.

Tangential sections of the stria were cut in an effort to provide a better three-dimensional demonstration of its epithelial circulatory pat-

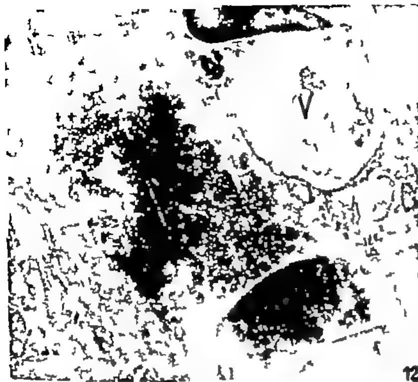


Fig. 12. Higher magnification of indicated area of Fig. 10. The small round densities within the granular membrane-bound mass indicate that leucocyte is degenerating. A large vacuole (*V*) containing few spherical particles can be seen in the epithelium. 29,000.



Fig. 13. Higher magnification of indicated area of Fig. 10. Ferritin granules (*F*) are obvious in the plasma of the vessel on the left. The one on the right has none which means flow is obstructed. A platelet (*P*) is approaching the endothelium. 29 000.

tern. Fig. 8 illustrates the normal complicated meshwork of strial vessels. Many right-angled anastomotic branches are seen. Fig. 9 is a similar preparation *from* an *adult Hedlund mink*. Only a few vessels remain. They ha e few branches. Serial sections revealed complete occlusion of many of these vessels.

Fibrin in blood clots was demonstrated in several *Hedlund specimens*. As illustrated in Fig. 11 typical finely striated strands of fibrin



Fig 14 Blood clot in the adult stria vessel. Many of the erythrocytes have begun to break down and lose their cell membranes. ×5700.

were often found alongside erythrocytes. White thrombi" as seen in higher magnification in Fig. 12 and platelets (Fig 13) were fairly common

Fig. 14 shows a mixture of degenerating erythrocytes and very dark plasma perhaps free hemoglobin in Hedlund stria near a [illegible]ous

When specimens from earlier stages were examined to find the earliest evidence of impaired circulation in Hedlund mink, endothelial swelling was found as early as two weeks after birth Fig. 15 shows a normal two week strial vessel. Fig. 16 is a comparable specimen from a Hedlund animal The lumen is almost completely occluded by a swollen endothelial cell. Serial sectioning showed that this represented the widest portion of this constricted segment. Under higher magnification abnormal cytoplasmic particles (Fig. 17) were noted in the cytoplasm of this swollen endothelial cell. They have some features in common with arbovirus particles Dr Jordi Casals, of the Department of Epidemiology Public Health, performed a series of screening hemagglutination inhibition serological studies which failed to demonstrate significant titres of any of the common varieties of arbovirus.

DISCUSSION

Thanks to the imaginative work of the Fowler & Fowler (1950) there has been much interest in the possible importance of "sludged blood" in otic abnormalities. Knisely (1941 1955, 1961), and his followers generated considerable discussion with reports of blood sludge as an important factor in numerous disorders (Bloch *et al* 1956) Heimbecker and Bigelow (1950) reported that generalized intravascular agglutination of erythrocytes followed trauma with accentuation by shock. David & Landau (1956) described characteristic nailbed and conjunctival vessel changes which they could recognize in 80% of patients with rheumatic fever or rheumatic heart disease Recently efforts have been made to refine observation methods. Zimmer & Demis (1964) used television cinematography to study human cutaneous vessels, and Brånemark *et al.* (1964) developed a tubed pedicle flap of skin from the arm which was fitted with a small chamber



Fig 15 Normal vessels of the three week mink stria vascularis. 4650



Fig 16. Hedlund stria vascularis at three week stage. The lumen is almost completely occluded by swollen endothelial cell (E). 4650.

permitting high quality photomicrography of circulation in small human vessels.

In an excellent study which avoided the cultism which has tended to complicate discussions of this topic, Ditzel (1959) performed a set of experiments which showed that intravascular agglutination of erythrocytes is a result of alterations of serum protein, particularly elevation of globulin and decrease of albumin. His results tended to support Fåhraeus (1921) concept that aggregation is most often harmless, but may sometimes act as a factor in the formation of thrombi. (He credits Coccius for first observing red blood cell clumping in 1852.)

In response to many stimuli, small blood vessels change as part of the inflammatory process. Clark & Clark (1935) published an excellent description of the alterations they observed in vessels of the tadpole tail They listed a progress of endothelial changes from momentary sticking of leucocytes to emigration of leucocytes and the final stage of actual extravasation of erythrocytes. Recently Majno *et al* (1961) have studied vascular responses to histamine and serotonin by light and electron microscopy They showed that colloidal HgS particles which ranged from 70 to 350 A leaked through the endothelium of affected vessels. These leaks were confined to



Fig 14 Blood clot in the adult stria vessel. Many of the erythrocytes have begun to break down and lose their cell membranes. 5700.

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pairment of circulation. The vascular network was much simplified in Hedlund stria with almost no anastomotic channels crossing between longitudinal vessels.

The stria vascularis of deaf Hedlund mink is atrophic. This atrophy is associated with a severe circulatory impairment which exaggerates the normal sluggishness of strial blood flow. Intravascular fibrin strands, white thrombi and adherent platelets interfered with flow of blood through these vessels enough to prevent entrance of small particles of ferritin. Many of the normal vessels comprising the network of strial blood supply disappear during maturation of Hedlund mink.

ZUSAMMENFASSUNG

Die Stria vascularis von tauben Hedlund-Nerzen ist atrophisch und ihre Gefässversorgung ist besonders träg. Die vaskuläre Anordnung ist primitiver als bei den normalen Tieren, denn viele normale verbindende Gefässe verschwinden während der Aufwachsung. Gefässtransport kann mit Ferritinpartikeln gut unter dem Elektronenmikroskop studiert werden. Injiziertes Ferritin drängt nicht in die Striagefässe von Hedlund-Nerzen ein. Intravaskuläre Koagulierung und Schwellung des Endothels wurden in diesen Gefässen bemerkt.

ACKNOWLEDGMENT

We are grateful to Dr Jordi Casals for performing series of arbovirus serological tests.

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OSTEOGENESIS IMPERFECTA

Light and electron microscopic studies of the stapes

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The light and electron microscopic findings in the stapes from a patient with osteogenesis imperfecta are correlated with the findings in the normal and otosclerotic stapes. In osteogenesis imperfecta there is a quantitative but not qualitative change of the bony tissue The histological findings in patients with osteogenesis imperfecta reported in the otosclerotic literature are reviewed. The conductive hearing loss in patients with osteogenesis imperfecta is occasionally due to an otosclerotic focus, in other cases to fibrous degeneration of the stapedial crura.

Osteogenesis imperfecta (o i.) is a fairly rare disease hereditary or sporadic, which affects mainly the bony tissue, but which may also involve other mesenchymal tissues (skin sclerae ments, and fascia) It manifests itself clinically in two types, a congenital and a tardive type, the latter setting in after the 2nd decade The bones are abnormally brittle and multiple spontaneous fractures—especially of the long bones—therefore common This is because the cortical layer of the bones and the trabeculae of the spongiosa are thin (McKusick 1960)

The aetiology is unknown, but a deficient differentiation of the osteoblasts is believed to be the reason why *normal compact bone does* not form (Wilton, 1932) It has not been possible to demonstrate changes in the calcium and phosphorus metabolism (Albright & Reifenstein, 1948)

The characteristic syndrome of blue sclerae, brittle bones with a tendency to fracture, and hearing loss of a conductive type is generally related to the names of van der Hoeve and de Kleyn who were the first to suggest, in 1918, that the hearing loss was due to otosclerosis. Since then several authors have discussed the possible relation between o.i and otosclerosis on the basis of histological studies (cf. Bretlau & Jørgensen, 1969 Opheim 1968) Most of the studies have been done post mortem on *temporal bones from patients having only o.i.* (as a rule, congenital more rarely tardive). Temporal bones from patients with o.i. have shown histological otosclerosis in the labyrinthine capsule in only 3 cases (Ruttin, 1922; Gimplinger 1926 Bretlau & Jørgensen, 1969) especially in the area of the oval window

Surgical treatment of the conductive hearing impairment in patients with o.i. has afforded a possibility of studying the removed bone histologically This applies to fenestration operations as well as to stapedectomies. As some of these patients have exhibited otosclerotic foci at the same time, the histological structure of the two diseases could be compared.

Since, however o.i. is a rare disease, the histological investigations of operation series are few and far between in otological literature.

On the basis of such histological studies Wullstein *et al* (1960) and Ogilvie & Hall

(1962) among others, have advanced the theory that o.i. and otosclerosis are based upon a common genetic abnormality and that otosclerosis is usually a local manifestation of o.i. This theory has recently been contested on the basis of clinical and histological studies (Altmann, 1962; Altmann & Kornfeld, 1967; Bretlau & Jørgensen, 1969; Opheim, 1968).

In spite of the conflicting views concerning the histological appearances of the two diseases, their clinical features are very much alike. Nevertheless, only a few patients with o.i. have been subjected to operation. Shambaugh found only 3 cases of o.i. in 2000 subjected to fenestration of the lateral semicircular canal. In all three he found grossly typical otosclerotic changes of the bony tissue at the anterior part of the oval window, but a histological study was not performed (Shambaugh, 1959). Chevance (1965) stated that only 8 cases of o.i. were found among 2500 stapedial operations during 3 years. This is presumably due to the rare occurrence of the disease, but perhaps also to some reserve in operating on patients with o.i., since apart from the conductive loss their disease often has an appreciable element of perceptive loss (Ruttin, 1922; Clerc & Déumier, 1958; Opheim, 1968).

Brickley (1947), Jelnes (1949), and Cremin (1952) were the first to report results of fenestration operations on patients with o.i., but none of them submitted histological proof of otosclerosis.

In 1958 Clerc & Déumier found otosclerotic changes in 3 patients with o.i. who had been treated by stapes mobilization. On the other hand, Sooy (1960) could find no gross evidence of fixation of the footplate in 4 patients with o.i. on whom he operated because of a suspicion of otosclerosis.

In 1960 Wullstein *et al.* reported the result of histological studies of bony pieces removed by fenestration operation from 2 patients with van der Hoeve's syndrome. Changes suggestive of otosclerosis were found in both, and the authors emphasized the histological similarity of the two diseases. They interpreted the otosclerosis as a result of a congenital defect in the osteoblasts. This was supposed to result in focal absorption of the bony tissue in the labyrinthine capsule, whereupon new bone formation occurs later.

Ogilvie & Hall (1962), studying the temporal bones from 7 cases of o.i., claimed to have observed otosclerosis as a local manifestation of o.i. However, the otosclerosis could not be confirmed histologically.

In 1963 Shea *et al.* reported on the results of stapedectomy on 6 patients with o.i. (9 ears). Only one case was examined histologically, and in this case Altmann found an otosclerotic focus at the tip of both stapedial crura. In every case much soft abnormal bone, covered by thick haemorrhagic mucoperiosteum, was noted in the stapes and oval window niche.

Altmann (1962) and Altmann & Kornfeld (1967) were unable to find otosclerosis in studies of the temporal bones of 5 patients with congenital o.i.

Andersen (1968), operating upon 3 patients with o.i., found typical histological changes of otosclerosis in only one.

Most recently Opheim (1968) has reported 4 cases in which mobilization of the stapes was performed in patients with o.i. He found no signs of gross otosclerosis in the stapes or labyrinthine capsule. On the other hand, he was the first to emphasize that the conductive hearing loss in patients with o.i. may be due to fibrous degeneration of the stapedial crura—as a result of generalized skeletal disease. This degeneration may result in fracture of the stapedial crura, compromising the conduction to the footplate. This finding is supported by Hall & Röhrt (1968) who found stapedial degeneration in the histological study of serial sections of the stapes from a patient with congenital o.i.

Recently, we had occasion to perform systematic histological studies on the stapes from a patient with o.i. tarda who underwent stapedectomy. These studies were supplemented by electron microscopic investigation of the bony tissue.



Fig. 1. Gross appearance of the removed stapes, showing the head, neck, and part of both crura of the stapes. Note the brittle transparent bony tissue with calcification (C). [illegible]

CASE REPORT

A woman, aged 60, with blue sclerae and a history of innumerable fractures. Since the age of 13 steadily increasing hearing loss in both ears without tinnitus or dizziness. No family history of hearing impairment. Hearing aid since 1946.

When first admitted in 1967 she had a bilateral, symmetrical, mixed perceptive–conductive hearing loss of 70–80 dB in the speech range. The perceptive component made up about 40 dB. Discrimination loss on the right 4%, on the left 16%. The ear drums were normal without Schwartze's phenomenon. She could not hear whispered voice, while conversational voice was just perceived close to the ear. Tubal and vestibular function normal.

In May 1967 stapedectomy by the method of Shea was performed on the *left*. Postoperatively the hearing was improved for 6 months, but thereafter deteriorated to its former level. Re-operation in January 1968 showed the prosthesis to be in the middle ear. Since the long crus of the incus was thin and lacked its tip, a tympanoplastic repair was carried out, but this did not improve her hearing much.

In January 1969 a Shea stapedectomy was



Fig. 2. Cross-section of the stapedial crura, showing an irregularly arranged bony tissue at the bottom of the picture and denser bony tissue at the top. No signs of otosclerosis. ×58

done on the *right*. Postoperatively there has been marked improvement for almost 6 months, the conductive hearing loss now being only 30–50 dB in the speech range.

X-rays (tomography with polytome) showed moderate narrowing of both oval windows with signs of a small otosclerotic focus in front of the left one. No signs of cochlear otosclerosis or closure of the round window (signed Rovsing). Other X-rays, of the temporal bones and chest, revealed halisteresis. Haematological and urinary investigations have shown no signs of changes in the calcium or phosphorus metabolism.

OBSERVATIONS

(*a*) *Gross*

On both sides the long crus of the incus was thinner than normal, almost atrophic. The stapedial crura on both sides were fractured, slender and disconnected from the footplate. The removed part of the stapes on the right, comprising the head and part of the crura, was plump, deformed, and almost pellucid (Fig. 1). On the figure it is possible to make out also cavitations at the neck of the stapes.

On both sides there was a thick layer of mucoperiosteum covering the oval window. Both footplates were fixed in the oval windows, but only on the left was there an otosclerotic focus anteriorly but not involving the oval window. The footplate itself on this side was very thick, while on the right it was nodular, porous, and more yellowish than usual in otosclerosis. When the right one was removed, in 4–5 pieces, it was found to be more brittle than is usual in otosclerosis.

The round window was normal on both sides.

(*b*) *Microscopic examination*

Only the *right* stapes was studied histologically. *Technique*. The stapes removed at the stapedectomy was fixed immediately in a solution of 2% glutaraldehyde mixed with cacodylate buffer, pH 7.2. After 24 hours at 4° C in this solution the stapes was decalcified in a 4.5% solution of EDTA 2 Na adjusted by NaOH to pH 7.2. After rinsing in Tyrode's solution and post fixation in 4% osmic acid OsO_4, the stapes was dehydrated and embedded in Epon as advocated by Luft (1961). Epon solution A and B is mixed at the ratio 7 : 3.

From the preparation block, survey sections of 1–2 μ were made for light microscopic studies. These sections were stained with 1% toluidine blue in a saturated solution of borax. Various sites were selected for electron micro-



Fig 3 Light microscopic appearance of the neck and head of the stapes showing cavities lined with middle-ear epithelium. No signs of otosclerosis. ×48.

scopic studies, and thereafter the entire stapes was cut into 1–2 μ sections to show the cavities in the neck. The ultrathin sections were collected on copper grids and contrasted with uranyl acetate and basic Pb citrate for 5 min at room temperature. The electron microscopic studies were performed with a Philips 100 B electron microscope.

Light microscopy

The sections through the stapedial arcade and the pieces of the footplate clearly showed the normal compact bone to have been replaced by more irregular coarsely fibrillated bony tissue of immature type.

Fig. 2 presents a cross-section through the crura. The bony tissue is loose, with fewer hav-

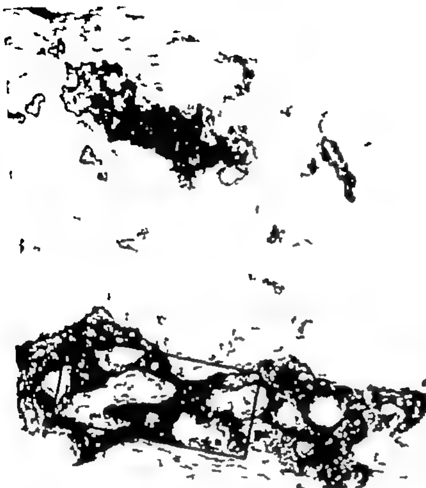


Fig 4 Part f the footplate showing porous, fragile bony tissue lying in dense connective tissue No signs of otosclerosis 57

ellated areas than normal. Part of the bony tissue shows numerous irregular resorption spaces containing connective tissue, alternating with areas of denser bone Only a few faintly organized Haversian systems were found in a few sections. Old fractures were not observed.

Fig. 3 illustrates a cross-section through the neck of the stapes. The bony tissue houses several large cavities lined with middle-ear mucosa.

The tissue pieces from the footplate (Fig 4) show marked fragility of the bone, major vascular cavities with connective tissue being scat tered in the osseous tissue. The bone has in several places preserved its lamellation, but without showing distinct Haversian systems. The osteocytes are typical, numerous and round to spindle-shaped, while there are but few osteoblast-like cells This bony tissue is surrounded by abundant connective tissue with scanty fibres and cells almost ensheathing the sparse pieces of bone Within this connective tissue, separated



Fig 5 Reichart anoptral-contrast (negative contrast) photomicrograph of the head of the stapes showing the disarrangement of the Haversian systems. 570.

the remaining bone, there is a thin, new formed, non-lamellated osteoid tissue containing only a few osteocytes

These bony changes were present also in the crura and head of the stapes, but in these sites the tissue showed a tendency to denser structure with several normal Haversian lamellar systems. This might indicate that partially normal re building of bone was taking place here. These sections also revealed cementing lines, separating bony tissue formed at different times.

The sections showed on the whole, only a few osteoblasts and no osteoclasts. In particular there was no otosclerotic tissue

Electron microscopy

The ultrastructure was studied in the areas framed in Figs. 2 and 4 In the bony matrix the normal regular arrangement of the collagen fibrils could not be traced. The arrangement was extremely irregular (Fig. 6) the collagen fibrils being in some areas short, broad bundles orientated in different directions, while in other sections they showed no orientation at all. The most characteristic finding was increased connective tissue and thinning of the collagen fibrils (Figs. 8 9) The striation of the fibrils was preserved and their thickness normal

The lacunae in the bony tissue were loosely encircled by fibres. Some contained a granular material and cellular debris (Fig. 7) In other respects the cellular structures were not altered as compared with normal bony tissue. The osteocytes in the lacunae were of uniform size and not more numerous than usual. Only a few cellular organelles were present, indicating that the osteocytes might be resting. The osteocytes showed no signs of lysis. On the other hand,



Fig. 6. Electron micrograph from the stapedial crura (the area indicated in Fig. 2) showing the derangement of the matri with cavities filled with debris and granular material. 9570.

there were occasional osteoblasts in the periosteum (Fig. 8) At this site the cell nuclei were typically peripheral in the cytoplasm which contained abundant endoplastic reticulum and mitochondria (Fig. 8) Osteoclasts were not observed. Numerous canaliculi penetrated the matrix which was also split up into numerous small cavities.

DISCUSSION

The normal structure of bony tissue and its transformation into compact bone depends upon osteoblastic and osteoclastic activity In patients with o.i. this structure is incomplete, the differentiation of the osteoblasts being deficient. On the other hand, the growth and differentia



Fig. 7. Electron micrograph from the stapedial crura (the area indicated in Fig.) showing an osteocyte (*O*). The cell has large nucleus with irregularly dispersed chromatin. Some granular material and a few mitochondria in the scanty cytoplasm. ×7700.

tion of the cartilage are normal (Follis, 1953). O.i. is essentially a failure of periosteal and endosteal membrane bone formation (Rubin, 1964).

In o.i. there is in the compact bone an abnormal distribution of mineral salts and of the arrangement of the collagen fibres (Engfeldt *et al.* 1954). This immature fibrillar bony tissue is reminiscent of the new formed cancellous bone found in newborns and children.

In normal bone new formation and destruction are going on all the time. Resorption spaces form and fill with new bony substance, making up new Haversian systems (osteons). On microradiography of normal bone (Amprino & Engström, 1952), therefore, the Haversian systems do not show uniform calcification, the oldest bone containing most calcium. On the other hand, microradiography of bony tissue from patients with o.i. (Engfeldt *et al.*, 1954) shows



Fig. 8 Electron micrograph from the stapedial footplate (the area indicated in Fig. 4). An osteoblast (*OB*) with endoplasmic reticulum is visible in the cytoplasm, lying in the loose bony matrix. 7830

the same mineralization in all sections and only a few Haversian systems.

The new formation of bone seen in o.i. is abnormal. Absorption of bone takes place, but the new-formed bone does not develop in distinct Haversian systems, showing instead an immature lamellated bony tissue.

These histological and biophysical studies of the bony tissue in patients with o.i. have been done on sections from the long bones. In the otological literature the findings in temporal bones, stapes, or bony tissue removed in fenestration procedures have been extremely varied. Some workers have found otosclerosis in the bony tissue, others otosclerosis-like tissue, while others again have been unable to find such changes. It had been hoped that simultaneous studies of bony tissue from patients with o.i.,



Fig 8 Electron micrograph from the stapedial foot plate (the area indicated in Fig. 4). Note the loose bony matrix with loose collagen fibrils (*CL*) and ample connective tissue (*CT*). 6760.

both from fractures in the long bones and from the stapes or labyrinthine capsule, might solve the aetiology of otosclerosis (Weber 1930 Frost, 1960) Unfortunately there is no proof that o.i. and otosclerosis are alike in anything but histological structure while the aetiology seems to differ

In a previous paper (Bretlau & Jørgensen, 1969) we have emphasized, as did Altmann (1962, 1967), that when otosclerosis and osteogenesis imperfecta are present in the same bony tissue, the two conditions may be distinguished histologically from one another

Our findings in the present patient confirm Opheim's observation (1968) that the conductive hearing loss in patients with o.i. may be due to osseous degeneration of the stapedial crura and need not necessarily be caused by otosclerosis.

Operation on the *left* ear of our patient in *1967 revealed an otosclerotic focus* which, however did not involve the oval window On the

other hand, the crura of the stapes were fractured and the footplate was considerably thicker than normal, a finding which has also been reported by others (Sooy 1960 Shea *et al.* 1963) However we lack histological study of the stapes removed from this side. Tomography of this ear at a later date confirmed the suspicion of an otosclerotic focus

On the other hand, the *right* ear has not shown, either at operation in January 1969 or at tomography any evidence of otosclerosis, and histological examination failed to reveal signs thereof in any section. On this side too the stapes was slender and fractured. The footplate was porous, delicate, and housed numerous small islets of mucosa. This is in keeping with the changes found in other parts of the skeleton in patients with o i. Chevance (1965) has reported the same findings in the stapes.

Tomography on the right ear showed a narrowed oval window However this narrowing may well be due to the very thick mucosa found on both sides at the oval window (Rovsing, 1969). Shea *et al* (1963) have made the same finding.

Our light as well as electron microscopic studies in this case have confirmed the well-known fact that the bony tissue in osteogenesis imperfecta exhibits quantitative, not qualitative changes as compared with normal bone The structure corresponds to normal osseous tissue but the appearances are more immature The fragile skeleton in o.i. is not due exclusively to the lack of normal compact bone, but also to the irregular arrangement of the fibrils in the organic matrix.

Comparison with the ultrastructure of the normal stapes (Reydon & Smith, 1968 Frank *et al* 1968 Chevance ɛt *al.*, 1969) reveals that the normal compact bone is replaced by a coarser irregularly fibrillated bone with an increased quantity of connective tissue

The structure of the bony matrix is reminiscent, in several respects, of the findings in otosclerosis but is much looser Another important difference relates to the cellular structures. The osteocytes did not show signs of the osteolysis which we have found previously in otosclerotic foci (Chevance *et al* 1969) This might indicate that the osteocytes do not play any major role in the bone resorption in patients with o.i. However it must be borne in mind that our material is from a patient aged 60 It would be more interesting to receive bony material from a younger adult, or better from a child with o.i. for electron microscopic investigation

After the reports of Opheim and of Hall & Röhrt from 1968 we reviewed the serial sections from our two previously published cases of o.i. (Bretlau & Jørgensen, 1969), but without being able to demonstrate any degeneration of the stapes

On the basis of our findings we deduce that the conductive hearing loss in patients with o.i. may be due to an otosclerotic focus, but that it may also be the result of a degenerative process in the stapedial crura.

We share Altmann's view (1962, 1967) that the histological findings in o.i. and otosclerosis are so different that they cannot support the view that o.i. and otosclerosis might be of the same basic aetiology

ZUSAMMENFASSUNG

Die licht- und elektronenmikroskopischen Befunde im Steigbügel von einem Patient mit Osteogenesis imperfekta sind mit den Befunden in normalen und otosklerotischen Steigbügeln verglichen geworden. Bei Osteogenesis imperfecta gibt es im Knochengewebe eine kvantitative und keine kvalitative Veränderung. Die histologischen Befunde bei Patienten mit Osteogenesis imperfecta, die in der otologischen Literatur mitgeteilt sind, werden in einer Übersicht gegeben. Die Schalleitungsschwerhörigkeit dieser Patienten ist in einigen Fällen von einem otosklerotischen Herd verursacht, in andern von einer Degeneration der Steigbügelschenkel.

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A model study in the diffusion chamber

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The diffusion of CO_2 through the tympanic membrane—removed after death—has been studied in a diffusion chamber under laboratory conditions. Thirteen measurements have been made and the mean value of CO_2-diffusion through the membrane per mm² and minute is 6.691 10^{-6} ml at atmospheric pressure. This makes 0.482 ml for the whole tympanic membrane (approx. 50 mm²) for 24 hours. When the relation in diffusion capacity between O_2, N and CO is known, the amount of oxygen and nitrogen diffusing at prevailing difference in partial pressure can be calculated to 0.3 and 1.26 microliters respectively. When the 24-hour ventilation through the Eustachian tube is 1 2 ml (Ingelstedt *et al.*) the values for oxygen and nitrogen are only 0.5–1 ‰ of the tubal amount. The figures calculated in the experiments seem to be higher than those *in vivo* where circulation with metabolic uptake of O and carrying away of N plays an important role. The normal tympanic membrane, therefore, does not seem to be able to contribute to the ventilation of the middle ear and the cellular system.

The purpose of this work has been to investigate firstly whether the respiratory gases diffuse through the normal tympanic membrane and—if so—to what extent; secondly whether the tympanic membrane plays a role in the normal ventilation of the tympanic cavity and cellular system besides the Eustachian tube. The investigation has been performed on tympanic membrane preparations in a diffusion chamber modified after Siesjö & Thews (1962).

TECHNIQUE AND METHOD

Temporal bones were taken from dead subjects with normal otoscopic findings and no history of ear diseases. The whole tympanic membrane with its annulus fibrosus was removed and two small circular parts with an area of 13.8 mm were cut out sharply from the pars tensa. The preparation was done as soon as possible after death, but for different reasons such as delay in the ward, transport to the pathological department etc. the measurements could not be made until approximately 6–8 hours later. During the preparation the tympanic membrane was continuously moistened with Tyrode solution to prevent drying and a nontraumatic technique being used. The preparation work was done under an operation microscope, magnification × 10. The parts cut out were immediately placed in Tyrode solution. One of the two parts was frozen in liquid propane and liquid nitrogen and after histological preparation with the conventional technique for cutting and colouring was examined for preserved histological structures and thickness.

The second part was examined in a diffusion chamber using CO_2. To a small glass chamber 3 cc in volume (determined in a microbalance using distilled water) was attached an Ingold pH-electrode with a ground neck (Fig. 1). On a level with this pH-electrode and connected to it was another small chamber made of plexiglass in which the circular piece cut from the tympanic membrane was placed (Fig. 2 *a* and *b*). The preparation of the membrane was placed so that the side lined with mucous membrane

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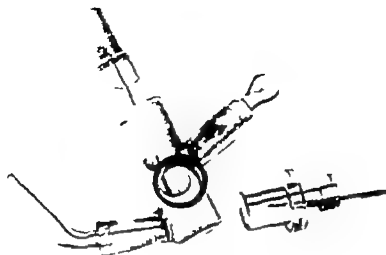
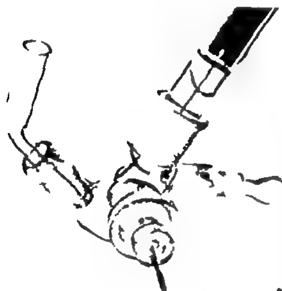


Fig 1 Ingold pH-electrode connected to a glass chamber containing a solution of 0.001 *N* $NaHCO_3$



a



b

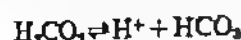
Fig 2 *and b.* Plexiglass chamber for tympanic membrane preparations.

faced the lumen of the glass chamber The chamber was filled with a 0 001-*N* solution of $NaHCO_3$ and in some cases with Krebs solution To avoid the buffering effect of the later solution, phosphate was excluded and it was made 0 001-*N* regarding $NaHCO_3$.

A reference electrode (Radiometer K 100) was immersed in a branch tube to the glass chamber and the electrode was connected to a pH-meter (Radiometer Copenhagen) Two plexiglass chambers were used differing from each other only in the diffusion area, which was 8 4 and 6 6 mm respectively The whole apparatus was immersed in a water bath, with a temperature of 37 C, controlled by a thermostat, and a magnetic stirrer was connected to the glass chamber The whole system was left to stabilize for 30 min pH was initially about 8 Fresh solutions of NaHCO and Krebs were used when possible and the electrodes and pH meter was calibrated against standard phosphate buffers (pH 6 840 and 7.385 at 37°C according to NBS) before every measurement. Pure carbon dioxide, heated to 37 C by passing through a copper coil in the bath was introduced into the plexiglass chamber and came into contact with the tympanic membrane. Measurements of pH were made at short intervals over a total period of 60–90 min.

Principle of determination

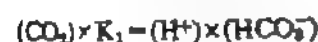
When CO_2, after diffusing through the tympanic membrane, comes into contact with the solution of $NaHCO_3$, H_2CO_3 is formed and rapidly dissociates to equilibrium



$$H_2CO_3 \rightleftharpoons H^+ + HCO_3$$

If we take the first imaginary ionisation constant of carbon dioxide

$K_1 = 10^{-6.352}$ we can write



$$(CO_2) \times K_1 = (H^+) \times (HCO_3^-)$$

The concentration of H -ions rises in direct proportion to the amount of CO_2 that has passed through the tympanic membrane, while pH decreases. At the same time the solution of $NaHCO_3$ also gets HCO_3-ions, but this amount is small and unimportant in comparison with that already present in the solution.

The amount of CO diffused through the tympanic membrane can be calculated from the equation

$$\Delta CO_2 = \frac{10}{10^{\;\;[illegible]}} \times \left[(H\;)_2 - (H\;)_1\right]$$

where $\Delta\ CO_2$ is the increase of solved CO_2 in mmol/ml of solution and $(H\;)_2$ and $(H\;)_1$ are the concentration of hydrogen ions at the end and the beginning of each measurement interval.

The volume, V of the glass chamber is 3 cc and the diffusion area, A is 8 4 and 6.6 mm respectively. This gives.

$$\frac{\Delta CO_2}{\Delta t} = \frac{V\ 22.3\ \ 10^{[illegible]}}{\Delta t\ \ A} \times \left[(H\;)_2 - (H\;)_1\right]$$

(22.3 is the mol volume of CO_2) thus being the amount of CO in ml that is diffusing through the tympanic membrane per unit of time and mm^2

MATERIAL

The material consist of 13 tympanic membranes. All of these have been removed post mortem from subjects with normal otoscopic findings

Table 1 *Tympanic membrane preparations*

No.	Thickness (μ)	Time after death (hrs)	Comment
1	44	7	Two different parts measured
	56		
	62	9	
3	—	9	Technical failure
4	100	7	Two different parts measured
	134		
5	124	9	Two different parts measured
	86		
6	—	6	Technical failure
7	114	11	
8	66	8	
9	406	6	Cutting dubious
10	—	8	Technical failure
11	54	10	
12	90	6	
13	90	6	

Mean thickness [illegible] μ (prep. no. 9 included).

and no history of ear diseases (Table 1) The time elapsing between death and the beginning of the measurements varied from 6 to 11 hours, the mean being 7 8 hours. The part to be examined histologically was frozen approximately at the same time as the diffusion studies were started, in some cases slightly earlier however The histological examination has been performed on only ten out of thirteen membranes because of different technical misfortunes in the preparation and cutting. The thickness given in Table 1 is the mean value of 5–8 microscopic measurements

RESULTS

The results are given in Table 2 The amount of pure carbon dioxide at atmospheric pressure that can diffuse through the tympanic membrane per minute and mm varies from $13\,652 \times 10^{-6}$ to $2\,452 \times 10^{-6}$ ml. The mean value is $6\,691 \times 10^{-6}$ the standard deviation 3.38×10^{-6} and the standard error of the mean 0.937×10^{-6}

DISCUSSION

In the exchange of gases in the middle ear nitrogen, oxygen and carbon dioxide tak part. There is a continuous process of gas taking

Table 2.

No.	Ml CO_2/min/mm² 10^{-4}	
1	..452	M = 6 691 10^{-4}
2	13.632	s.D. = 3.38 10^{-4}
3	3 118	s.E. = 0.937 10^{-4}
4	8.050	
5	10.851	
6	9.034	
7	6.462	
8	4.458	
9	9 491	
10	..931	
11	4.3..4	
12	5.898	
13	6.222	

absorbed into the surrounding tissues and this volume is compensated by air entering through the Eustachian tube if it is functioning normally Under normal conditions, the Eustachian tube is closed but opens at swallowing, yawning etc. This happens quite frequently and no notable underpressure will develop in the middle ear and the cellular system But the situation is different if the tub- is not functioning normally and only opens at high pressures in the rhinopharynx—the Valsalva manoeuvre—as may happen in diseases such as the common cold. Hence there is a feeling of "locking" which indicates a slowly rising underpressure in the sed cavity of the ear The Eustachian tube ction also varies according to body position (Ingelstedt *et al* 1967 Rundcrantz, 1969) and this can be accentuated in some people

We are thus dependent on an ad-quate ventilation of the middle ear system for a normal hearing capacity

The middle ear can be compared to an open, non ventilated gas-pocket according to Raba (Fig. 3) in connection with the atmosphere through the Eustachian tube Carbon dioxide will rapidly come into equilibrium with the surrounding tissues and aft r that there is no net diffusion of this gas Oxygen and nitrogen, on the other hand, are continuously absorbed and restored through the Eustachian tube. It is of some interest to find out whether the tympanic membrane can—in any way—take part in the gas exchange between the ear and the surrounding atmosphere and if so which role it can play quantitatively in comparison to the tubal ventilation. Investigations with xenon[133] (Riu *et al* 1966) do not indicate an exchange of any importance but this has not yet been established for the respiratory gases.

In this work carbon dioxide has been used. The reason is that CO_2 affords the best and easiest laboratory possibilities and thus has a certain advantage over a direct study with oxygen and nitrogen Since th- relation of CO to O_2 and N is fairly stable as regards solubility and the diffusion coefficients, information about O_2 and N can be gained from experimental data with CO

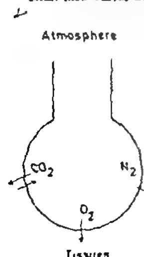


Fig 3 Open non ventilated gas-pocket according to Raba.

Table 3 *Gas composition in the middle ear according to Melville Jones and Riu, Flottes Bouche and Le Den*

	N	O_2	CO_2
Melville Jones			
Vol.	84	9	7
Partial pressure mm Hg	598.9	64.2	49.9
Riu et al.			
Vol.	85	9.5	5.5
Partial pressure, mm Hg	606	67 7	39 2

The partial pressures calculated on 713 mm Hg, i.e. 760 mm—the partial pressure for water vapour.

The gases in the middle ear system have been studied by Matsumura (1955) Melville Jones (1961) Riu *et al* (1966) among others (Table 3) The partial pressure of nitrogen in the middle ear can be calculated to approximately 600 mm Hg and that of oxygen to approximately 66 mm Hg at an atmospheric pressure of 760 mm Hg

The difference between the gas pressure in the middle ear and the surrounding tissues constitutes the "driving pressure for the diffusion of the gases in question

$$P_{ear\,N_2} - P_{atm\,N_2} = P_{diff\,N_2}$$

$$P_{ear\,O_2} - P_{atm\,O_2} = P_{diff\,O_2}$$

The partial pressure of nitrogen in the surrounding atmosphere is 573 mm Hg at 760 mm Hg atm. pressure, which is *less* than that in the middle ear A diffusion of nitrogen from the ear to the atmosphere should therefore be possible with a driving pressure of approximately 30 mm Hg.

$$P_{ear\,N_2} - P_{atm\,N_2} \approx 30 \text{ mm Hg}$$

The situation for oxygen should be the reverse. The partial pressure in the middle ear is about 66 mm Hg and the partial pressure in the atmosphere is about 140 mm Hg (responding 20 vol%) at 760 mm Hg.

$$P_{atm\,O_2} - P_{ear\,O_2} \approx 74 \text{ mm Hg}$$

A driving pressure of approximately 74 mm Hg should exist from the atmosphere to the middle ear The partial pressure for carbon dioxide in the ear is about 45 mm Hg (Table 3) and in the atmosphere nearly zero.

$$P_{ear\,CO_2} - P_{atm\,CO_2} \approx 45 \text{ mm Hg}$$

A diffusion of CO_2 from the ear to the atmosphere may be possible. The tympanic membrane has a diameter of approximately 9 mm. The area is about 57 mm² If the handle of the malleus is excluded there are about 50 mm² left as a diffusion area.

In 24 hours, pure carbon dioxide to an amount of 0 482 ml could pass the tympanic membrane at 760 mm Hg.

$$6.691 \times 10^{-6} \times 50 \times 60 \times 24 = 0\,482 \text{ ml}$$

At P_{diff} 45 mm Hg 0 030 ml = 30 microliters could pass.

According to Handbook of Respiration (1958) the relationship between the diffusion coefficients of O and N_2 is 1 0 46 and of O_2 and CO_2 1 36 calculated for connective tissue and at 20 C. The diffusion coefficient of the gases rises 1% per C and the relationship is the same at 37 C. The tympanic membrane consists largely of connective tissue and the figures above can be considered as representative The diffusion capacities between the respiratory gases are thus approximately

CO_2 O_2 N = 70 2 1

Pure oxygen at atmospheric pressure could then pass to an approximate amount of.

$$\frac{0.482}{35} = 0.0138 \text{ ml}$$

Pure nitrogen could under the same circumstances pass to the approximate amount of.

$$\frac{0\,482}{70} = 0.0069 \text{ ml}$$

If P_{diff} O_2 is about 66 mm Hg this makes 66/713 of the atmospheric pressure (760 mm — the partial pressure of water vapour) and

$$\frac{0\,0138 \times 66}{713} = 0\,00126 \text{ ml} = 1.26 \text{ microliters}$$

could diffuse through the tympanic membrane every 24 hours.

If P_{diff} N is about 30 mm Hg at 760 mm Hg

$$\frac{0\,0069 \quad 30}{713} = 0\,0003 \text{ ml} = 0.3 \text{ microliters}$$

could pass the membrane every 24 hours

The total exchange through the tympanic

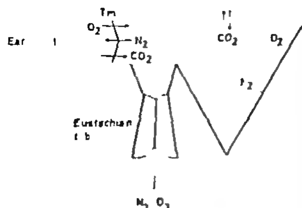


Fig 4 Gas exchange in the middle ear and cellular system.

membrane should be 30 + 0.3 + 1.26 = 31.56 microliters/24 hours. Of this, 30.3 microliters (CO_2+N_2) pass from the ear to the atmosphere and 1.26 microliters (O_2) from the atmosphere to the ear (Fig. 4) The carbon dioxide in the ear is, however in constant equilibrium with the surrounding tissues and will not contribute to the net ventilation of the ear Oxygen and nitrogen diffuse in the reverse direction and only 1.26 − 0 3 = 1 microliter should pass from the atmosphere to the ear

The total amount of gases passing through the Eustachian tube in 24 hours is about 1–2 ml (Ingelstedt *et al.* 1967) and the amount of diffusing to the ear through the tympanic brane is only 0.5–1 ‰ of the tubal ount. According to this, the normal tympanic membrane is unlikely to play any role in the ventilation of the middle ear and the cellular system. If we have thin and extensive scars in the tympanic membrane the situation might eventually be more favourable but this has not been studied. The investigation has been performed in tympanic membrane preparations. When there is no circulation the values are not quite relevant for the situation *in vivo* The diffusion of e.g. oxygen in the living individual is further complicated by a metabolism with consumption of oxygen. One dares to say however that the situation *in vivo* is not more favourable for the gas exchange of O_2 and N_2 and the approximate values calculated above should be lower even if the tympanic membrane is thin and sparsely vascularised in its central parts The investigation has been performed at 37 C. The temperature in the middle ear is about 37 C but is lower in the ear canal. A difference in temperature exists between the experimental and *in vivo* situations, but this is not likely to change the figures calculated very much, and this difference is not favourable to the *in vivo* situation. The histological examination did not show any autolysis or structural changes, but the fact that the measurements have been made some time after death must of course be remembered.

Zusammenfassung

Die Diffusion von Kohlendioxyd durch normale, postmortal präparierte Trommelfelle wurde in einer Diffusionskammer an 13 Präparaten studiert. Die durchschnittliche Diffusion von CO_2 beträgt bei atmosphärischem Druck 6.691 × 10 ml per mm² und Minute. Dieses würde auf das ganze Trommelfell und auf 4 Std. umgerechnet 0.482 ml ergeben. Die Diffusionsmengen von Stickstoff und Sauerstoff im Verhältnis zum CO sind bekannt, nämlich approximativ 1 : 79 Die Menge N_2 und O_2, die bei einem normalen Luftdruck von 760 mm Hg und der gegebenen Partialdruckunterschied zwischen Mittelohr und der umgebenden Atmosfäre durch das Trommelfell diffundieren könnte würde also 0,2 bzw 1,26 l betragen. Da der tägliche Luftaustausch über die Ohrentrompete auf 1 2 ml berechnet worden ist, würden die gesamten Diffusionswerte 0,5–1 ‰ der Tubarventilation ausmachen. Die *in vitro* erhaltenen Werte übersteigen wahrscheinlich die *in vivo* Werte da bei erhaltenem Blutkreislauf ein Verbrauch von Sauerstoff und Abtransport von Stickstoff vorkommt. *Auf Grund* dessen kann man nicht annehmen, dass das normale Trommelfell an der Durchlüftung des Mittelohrs oder Zellensystems beteiligt ist.

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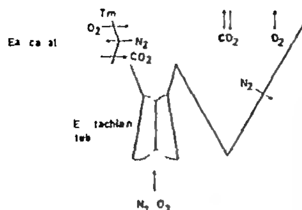


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mals. The technique for recording the cochlear potentials, cochlear microphonic (CM) action potential in the auditory nerve (AP) and endocochlear potential (EP) in the basal turn has been fully described in other papers (Tasaki *et al.*, 1952 Konishi *et al.* 1961 and Konishi *et al.* 1966) Tone bursts were used as sound stimuli.

Endolymph collection and its analysis

The techniques of sample collection and analysis of endolymph used in this experiment were essentially the same as those described elsewhere (Mendelsohn & Konishi, 1969) A double-barreled microelectrode was prepared, the tip of which was approximately 5 to 10 micra. One barrel was used for collection of the endolymph and the other for recording dc potential. The double-barreled micro-pipette was inserted into the scala media through the spiral ligament. When EP was recorded at the tip of the recording barrel, the fluid was gently sucked into the sample-collecting barrel. The sample was then expelled into 2 ml of triple distilled water The spectrophotometric determination of sodium and potassium concentration of the sample were made following the technique described in detail by Rogers & Chou (1966 *a*) To determine the volume of the sample, an equal amount of KC1 solution of known concentration was expelled into 2 ml of distilled water and the dilution factor was calculated by measuring the final concentration of potassium with the flame spectrophotometer

The perfusion technique and test solutions

The perfusion technique used in this experiment was fully described in other papers (Konishi *et al.*, 1967) Also the arrangement and procedure for injection of a test solution into the scala media were described in detail elsewhere (Konishi *et al.*, 1966). Approximately 5 microliters of the test solution per minute were introduced into the perilymphatic space The period of injection of a test solution into the scala media was 10 to 15 min and its total amount was estimated as 0.5 to 0 7 microliters.

The test solutions employed for the perfusion of the perilymphatic space were 10^{-4} to 10^{-8} *M* ouabain dissolved in Ringer Locke's solution (m*M* NaCl 147 KCl 5 6, $CaCl_2$ 2.16, $NaHCO_3$ 2.40) Sodium-free Ringer Locke's solution was made by substituting choline chloride 175 m*M* for NaCl in the above solution. In cases of injection into the scala media ouabain was dissolved in a solution containing one part of Ringer-Locke's solution and nine parts of isotonic KCl solution and its concentration was 10^{-3} and 10 *M*

RESULTS

Alteration of Cochlear Potentials

Perfusion with normal solutions

It has been shown by us (Konishi & Kelsey 1968 *a*) that the cochlear potentials did not change appreciably during the perfusion, when Ringer Locke's solution was introduced into the scala tympani at the rate of perfusion described in Methods. Similar data have been reported by Tasaki *et al.* (1952) and Moskovitz & Gannon (1966)

When potassium-rich Ringer Locke's solution was injected into the scala media at a constant rate of injection EP remained unchanged and CM was slightly depressed during the micro-injection. As reported by us (1968 *b*) the temporary depression of CM was 90–70% of the original value. CM showed complete recovery within 10 min after the end of the micro-injection. AP usually did not show any detectable changes during or after injection.

Treatment with ouabain

1 *Changes in EP* With 10 *M* ouabain Ringer Locke's solution in the scala tympani EP began to decline 3 to 5 min after introduction The depression of EP reached about half of the original magnitude at the end of the perfusion. In almost all cases EP showed no recovery even if ouabain solution was washed out with normal solutions (Fig. 1) The mag-

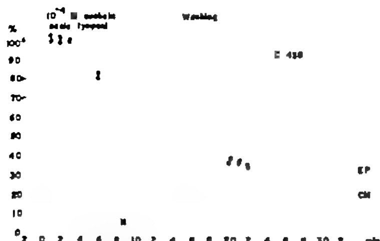


Fig 1 Changes of EP CM and N_1 during and after perfusion of scala tympani with 10^{-4} *M* ouabain solution.

nitude of EP ranged from 30 to 10 millivolts 1 hour after perfusion. None of our cases showed negative EP at the end of the observation period The depression of EP was still observed to a lesser degree with 10^{-5} *M* ouabain solution in the scala tympani. Concentrations of ouabain lower than 10^{-6} *M* did not depress EP when introduced into the scala tympani. EP remained unchanged or showed a slight increase during the perfusion. This increase of EP cannot be attributed to an increase of hydrostatic pressure of the scala tympani during the perfusion, because rapid perfusion of the scala tympani always counteracts this increase of EP

In order to compare possible differences in the effect on EP of the perfusion of scala tympani and scala vestibuli, 10^{-4} *M* ouabain solution was injected into one or the other of these scalae during one minute Our results showed no significant differences in the behavior of EP in either case (Fig. 2).

When 10^{-3} or 2×10^{-3} *M* ouabain solution was injected into the scala media, EP did not show immediate changes. EP exhibited a slight increase during the injection which, however did not exceed a 10% increase EP returned to its original magnitude and was slightly depressed during 30 min observation period after the injection (Fig. 3)

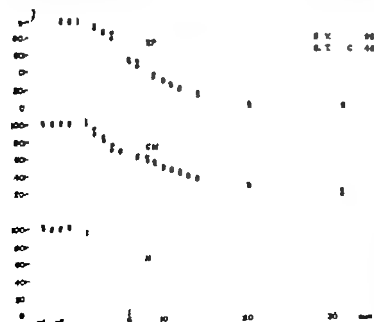


Fig 2 Comparison of the effect on cochlear potentials of perfusion of scala tympani and scala vestibuli. Test solution: 10^{-4} *M* ouabain duration of perfusion, 1 min, open and filled circles scala tympani and scala vestibuli respectively as recorded from two different animals.

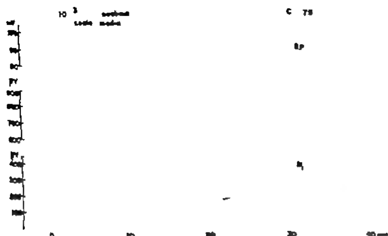


Fig. 3. Changes in EP, CM and N during and after the injection of 10^{-4} *M* ouabain into scala media.

2. *Changes in AP.* The most rapid change observed after the perfusion of the scala tympani with 10^{-4} *M* ouabain Ringer Locke's solution was a progressive decline of AP as shown in Fig. 1. Even if the ouabain solution was again replaced with normal Ringer-Locke's solution, there was no reappearance of AP. With 10 *M* ouabain solution in the scala tympani, AP decreased its magnitude to 50% at the end of the perfusion and was barely visible 5 to 10 min after the end of the perfusion. Washing of the scala tympani with normal Ringer Locke's solution was effective in restoring AP when carried out early. But no full recovery of AP was observed in our cases. Concentrations of ouabain lower than 10 *M* did not depress AP when introduced into the scala tympani. In some cases AP showed a slight increase after the perfusion.

As shown in Fig. 2, AP disappeared 6 min after the beginning of the perfusion, when 10 *M* ouabain solution was injected into the scala tympani during one minute. The effect of the perfusion of the scala vestibuli was slightly less in degree than that of perfusion of the scala tympani. On the other hand, AP was not affected when 10 or 2 × 10 *M* ouabain solution was injected into the scala media as shown in Fig. 3.

3. *Changes in CM.* CM showed a progressive decline during and subsequent to the perfusion of the scala tympani, when the concentration of ouabain was higher than 10 *M* (Fig. 1). With 10 *M* ouabain solution in the scala tympani the depression of CM was found to be approximately 90% at the end of the perfusion and CM continued to drop even after washing with normal solution. The input-output curve of CM recorded 15 minutes after the end of the perfusion with 10^{-4} *M* ouabain

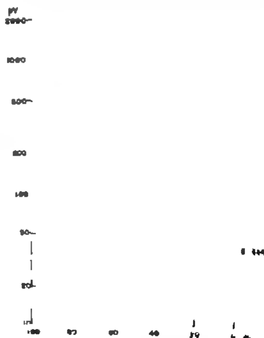


Fig. 4. Input-output relationship of CM before and after the perfusion of scala tympani with 10 *M* ouabain during 10 min. Open circles before, filled circles after the perfusion.

indicates that maximum output of CM was greatly depressed and implies dysfunction of the hair cells (Fig. 4) The perfusion of the scala tympani with concentrations of ouabain lower than 10 $^{-6}$ *M* did not result in a marked depression of CM The slight depression of CM observed during and after the perfusion was also seen in the control group as mentioned above and therefore the depression within this range of concentration cannot be attributed to the specific action of ouabain.

When 10 $^{-3}$ *M* or 2 × 10 $^{-3}$ *M* ouabain potassium-rich Ringer Locke s solution was injected into the scala media, CM began to decrease during the injection Maximum loss of CM ranged from 30 to 50% After injection, CM showed partial recovery in most cases, as shown in Fig. 3 With 5 × 10 $^{-4}$ *M* ouabain in the scala media, the maximum depression of CM was approximately 70% of the original magnitude.

Treatment with sodium-deficient ouabain

If the above mentioned effect of ouabain on the cochlear potentials is produced by the inhibition of sodium transport, lowering the concentration of sodium in the perilymph should minimize the action of ouabain A series of experiments was carried out, in which sodium was replaced by choline

As we reported previously (Konishi & Kelsey 1968 *a*) sodium lack in the perilymph caused reversible depression of AP but CM and EP were not seriously affected. The perilymph in the scalae vestibuli and tympani was replaced with choline Ringer Locke s solution until responses became stable Choline Ringer Locke s solution with 10 $^{-5}$ *M* ouabain was then introduced into the perilymphatic space during 10 min. Again, washing was carried out with normal Ringer Locke s solution.

Fig. 5 illustrates typical behavior of EP and CM in an animal pre-treated with choline Ringer Locke s solution and in a control Less depression of CM and EP was observed during the introduction of choline Ringer Locke s solution with 10^{-5} *M* ouabain than when normal



Fig 5 Effect of sodium deficiency in test solution on the response decline induced by 10^{-5} *M* ouabain. o, perfusion with choline Ringer's solution containing 10^{-5} *M* ouabain normal Ringer's with 10^{-5} *M* ouabain.

Ringer-ouabain solution was used. The recovery of EP was effected promptly by washing with normal Ringer Locke s solution and CM did not show any progressive decline during the period of washing. As AP disappeared during the period of perfusion with choline Ringer's solution, it was impossible to compare the changes in AP caused by sodium deficient ouabain solution with those in controls. However there was a clear cut difference in the recovery process of AP during the washing period.

Alteration of Endolymph Composition

The test solution was introduced into the scala tympani during 10 min. A sample of endolymph was collected 15 min after the end of the perfusion. The pseudothresholds of CM and AP as well as their input-output functions

Table 1

Animal no.	Threshold shift (dB) CM 8 KHz	1 KHz	0.25 KHz	AP (8 KHz)	EP (mv)	Endolymph K (mEq/l)	Na (mEq/l)
(1) *Control solution in scala tympani*							
571	1	5	9	8	80	152.6	1.2
581	3	7	6	7	85	148.7	5.7
607	3	4	0	6	85	144.0	3.7
612	−3	5	−4	4	70	147.0	2.2
613	1	1	0	3	80	156.0	2.4
Mean	1	4.4	2.2	5.6	80	149.7	3.0
(2) *10^{-5} M ouabain in scala tympani*							
562	6	2	22	65	35	156.6	2.7
565	9	8	12	80	46	153.9	5.9
566	5	2	10	39	33	148.5	7.3
606	3	2	1	40	40	155.5	10.5
(3) *10^{-4} M ouabain in scala tympani*							
555	16	13	15	95	25	153.2	17.1
558	24	11	10	81	6	141.8	19.8
561	15	9	6	90	16	131.2	13.5
575	15	9	4	100	9	147.2	4.7
582	8	8	6	96	16	107.9	9.7
583	8	9	8	87	22	114.8	12.0
615	10	10	9	93	30	144.0	14.8
617	7	14	8	87	24	128.0	13.8
Mean	12.9	10.4	8.3	91.1	18.5	133.8	13.2

Zero dB represents the mean threshold of CM or AP taken before the perfusion.

were measured before and 10 min after the end of the perfusion, and EP was recorded when the double-barreled pipette was inserted into the scala media. As shown in Table 1 administration of control solution into the scala tympani produced little change in EP or threshold of CM and AP. The potassium and sodium concentrations in the endolymph were 149.7 ± 4.2 mEq/l and 3.0 ± 1.6 mEq/l respectively. There were no significant differences between these values and those obtained in non-treated normal guinea pigs (Mendelsohn and Konishi, 1969).

When the scala tympani was perfused with 10^{-} M ouabain Ringer's solution, EP dropped considerably as mentioned in the previous section. The threshold of N was elevated to about 50 dB 15 min after the end of the perfusion. However the potassium and sodium concentration of the endolymph were found to be 153.6 ± 3.1 mEq/l and 6.6 ± 3.2 mEq/l respectively when the sample was collected 15 min after the perfusion. The statistical treatment did not show any significant deviations from the control values at the level of 5%.

Increase of concentration of ouabain to 10^{-4} M caused marked depression of the cochlear potentials at the moment when the sample of the endolymph was collected. The chemical analysis of the endolymph revealed considerable increase of sodium and decrease of potassium content as shown in Table 1. The mean value of sodium content was 13.2 mEq/l and its standard deviation was 4.3 mEq/l. This value was statistically significantly different from the control at the level of 1%.

DISCUSSION

Action of ouabain

There is ample evidence that the pharmacological effects of ouabain are specific. Schatzmann (1953) reported that cardiac glycosides (like

strophanthidin) in low concentrations act to inhibit active sodium and potassium transport. Caldwell & Keynes (1959) showed that ouabain inhibits the active extrusion of sodium from the giant axon of the squid only when it is applied to the external surface of the membrane Although other actions of cardiac glycosides have also been reported, e.g., inhibition of transport of sugars and amino acids, effects on excitation-contraction coupling in cardiac muscles (Glynn, 1964), cardiac glycosides have been used to distinguish between active and passive transport across the cell membrane. Therefore there is good reason to assume that ouabain introduced into the cochlea exhibits its specific inhibition of cation transport.

The depression of the cochlear responses is irreversible even after the perfusate containing ouabain is removed by washing with Ringer Locke's solution. In most cases these responses continue to diminish during and after the washing. Only with concentrations lower than 10^{-8} M did the cochlear potentials show delayed and partial recovery after washing. It may be that ouabain is irreversibly bound to its site in the cell membrane or that ouabain causes leakage of sodium chloride and water during inhibition of transport. This progressive depression of the cochlear potentials could be ...ially prevented by lowering sodium concentration in the perfusate. Since this perfus... lowers the electro-chemical gradient of sodium across the cell membrane, this fact seems to imply that the inhibitory effect of ouabain on the cochlear activity is caused by interference with sodium transport.

As cardiac glycosides do not affect passive transport, sodium and potassium move toward equilibrium between the perilymph and endolymph Therefore alternation of the ionic concentration of the endolymph after treatment with ouabain can be attributed to the passive diffusion of cations across the cochlear partition. This is also supported by the fact that similar changes in ionic composition of the endolymph have been produced by locally induced anoxia or by death, because the active transport is stopped by anoxia or after death (Rogers & Chou, 1966 b Mendelsohn & Konishi, 1969).

Rate of action

The rate at which ouabain depresses the cochlear potentials is determined by the time which is necessary for ouabain to diffuse to its site of action and to manifest its pharmacological effect. Frank & Goldsmith (1967), reported that the electroretinogram was abolished in 6 min with 10^{-4} M ouabain and depressed to 90% with 3×10^{-6} M ouabain. The application of 10^{-5} M ouabain to the giant axon of the squid promptly slows the active extrusion of sodium but there is no detectable change in the resting or action potential (Caldwell & Keynes, 1959) Birk (1963) found that digoxin in various concentrations greater than 1.3×10^{-6} M required at least half an hour to produce transmission block in the fine motor terminals of frog skeletal muscles. These facts can be explained by the fact that the sensitivity of different animals to ouabain varies within wide limits (Skou, 1965) and also the anatomical relation between the surface area and the volume of the structures affected differs in various species.

Site of action

Identification of the sites of action of ouabain is not possible from our experimental data. Not only ouabain but also other electrolytes such as KCl affect AP faster when introduced into the scala tympani than into the scala vestibuli. However there was little difference in the behavior of EP and CM between perfusion of scala tympani and scala vestibuli This finding poses an interesting question concerning the function of Reissner's membrane. Rauch et al. (1963) reported that radioactive potassium enters the endolymph through Reissner's membrane at a greater rate than radioactive sodium (Lawrence et al 1961) also suggested from their electronmicrographs that Reissner's membrane can serve as a selective diffusing tissue.

If this is the case, the introduction of ouabain into the scala vestibuli should have a more severe effect on CM than when placed in the scala tympani, as CM is sensitive to any alteration of sodium concentration of the endolymph. Recently Iinuma (1967) reported high membrane ATP-ase activity in the stria vascularis and spiral ligament, which is closely related to the active transport. Although our results did not give us any direct identification of the site of action of ouabain, they seem to imply that changes in both ionic content of the endolymph and EP are due to selective blocking of active transport by ouabain in the stria vascularis.

RESUME

L'effet de la ouabaine sur les potentiels cochléaires et la composition de la fluide endolymphatique à été etudié sur les cobayes. L'introduction de ouabaine dans l'espace périlymphatique à resulté dans un dépression sévère des potentiels cochléaires, et une augmentation du sodium ainsi qu'une diminution du potassium dans l'endolymphe. En réduisant la concentration du sodium dans la périlymphe nous diminuons l'action de la ouabaine. Quelques généralités concernant les rapports entre les potentiels cochléaires, l'endolymphe et le transport des ions actifs furent discutés.

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THE INFLUENCE OF QUININE ON THE AMPULLAR RECEPTORS OF THE ISOLATED POSTERIOR SEMICIRCULAR CANAL OF THE FROG

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The action of quinine hydrochloride on the ampullar receptors of the isolated posterior semicircular canal has been studied, using electrophysiological methods. In the Tyrode solution added with quinine hydrochloride both the response to stimulation of the ampullar receptors and the spontaneous acti ity were promptly inhibited at 2.5 γ/ml of quinine hydrochloride and almost completely blocked t 20 γ ml. Following irrigation of solution to wash out the quinine the response to stimulation of the ampullar receptors and the spontaneous activity were sl ghtly recovered.

It has been well known that some drugs such as quinine salicylates, nicotine, arsenics, streptomyces antibiotics and others may cause toxic lesions of the inner ear Enormous studies on influence of dihydrostreptomycin upon the r ear using electrophysiologic, histopatho- c, electron microscopic or biochemical methods, have been reported. These ototoxic me dicaments have proved to cause degeneration of the receptor cells, ganglion spirale and nerve fibres

The author obtained the potential from an isolated posterior semicircular canal of frog to obtain some data on the changes of the potential under direct influence of quinine hydrochloride on the receptor cells

METHOD

The posterior semicircular canal of frog (Rana esculenta) together with the corresponding ampullar nerve is isolated in Tyrode solution under microscopic control A thin glass pipette is introduced into the posterior end of the isolated canal The pipette is connected to a micrometer syringe filled with Tyrode solution. By rotating the micrometer syringe at certain degrees, a controlled flow of fluid in the canal and in the ampulla can be obtained (Fig. 1) About 0.15 μl of the fluid in the canal is ampullofugally aspirated by a trigger this manipulation is used as a standard stimulus for the preparation.

The cut end of the ampullar nerve is sucked to the opening of a second pipette provided with two platinum electrodes, by means of which the action potentials are led-off and observed on a Braun tube oscilloscope. The response to the stimulation has been evaluated by counting the number of spikes over 50 μV discharged within 7 sec immediately after the stimulus, utilizing an electronic counter

Since the response of the semicircular canal becomes constant at about 20 min after the dissection, the value at 20 min was adopted as the initial value Then one minute after the addition of quinine hydrochloride to the bathing fluid the response was determined Measurements were repeated every 6 minutes 43 min after the administration of quinine the preparation was washed repeatedly with fresh Tyrode solution and further determinations at 6 min intervals were made

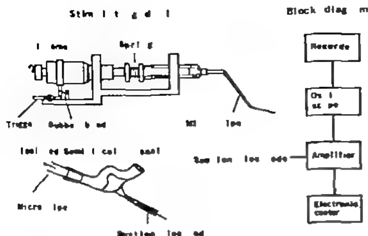


Fig 1

Concentrations of quinine hydrochloride used in this experiment were 2.5 γ/ml, 10 γ/ml and 20 γ/ml in the bathing Tyrode solution.

At least five experiments have been performed on different preparations for each concentration of quinine hydrochloride A control was performed for each group of treatment in Tyrode solution without quinine.

RESULTS

Response of the preparation in ordinary Tyrode solution (Control)

Immediately after dissection the preparation showed much less activity but 20 min after dissection 1000–2000 spikes could be counted within 7 sec on the stimulation. This initial activity was followed by a slight ascending activity After changing the Tyrode solution at 45 min the number of the spikes further increased up to 125% of the initial activity (100%) during the course of the experiment (up to 85 min) the number of the spikes did not decrease

Influence of quinine hydrochloride on the response

At levels of concentration under 1γ ml of quinine hydrochloride in Tyrode solution the influence of the drug on the posterior semicircular canal was not remarkable 2.5 γ/ml concentration was enough to inhibit the activity immediately after quinine administration the number of spikes decreased to 1158 (72%) from the initial activity of 1589 (100%) after 19 min, and to 1017 (64%) after 43 min.

By administration of 5 /ml the inhibition was still more marked. Initial activity was 1914 (100%) after 19 min being 876 (45%) and after 45 min 793 (42%)

At a 10 γ/ml concentration of quinine, initial activity was 1682 (100%) after 19 min 500 (29%) and after 43 min 441 (26%)

At a 20 γ/ml concentration of quinine, initial activity was 1490 (100%) after 19 min 418 (28%) and after 43 min 345 (16%)

Recovery with change of Tyrode solution

After 45 min in quinine solution the bathing Tyrode solution was changed several times to wash off the quinine as completely as possible, and further estimations were made

In the group which had received quinine in concentrations of 2.5 ml and 5 γ ml, recovery of the electric activity showed approximately 70 of the initial activity

In groups administered with quinine in concentrations of 10 ml and 20 γ ml the recovery rate was only 45 and 49% respectively

The recovery following change of Tyrode solution was slow but after 4 min the maximum was reached and th level was maintained thereafte

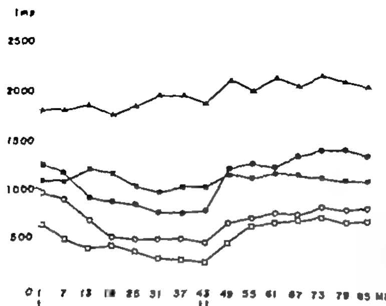


Fig 2 Action of quinine hydrochloride on the ampullar receptors' response to stimulation.

Imp counted spikes within 7 sec. Addition (↑) and removal (↑↑) of the quinine. ▲–▲ control; ■ ■ quin. hydrochlor 2.5 γ/ml; ● ● quin. hydrochlor 5 γ/ml; ○ ○ quin. hydrochlor 10 γ/ml, □ □ quin. hydrochlor. 20γ/ml.

The influence of quinine hydrochloride on the spontaneous discharge

The number of spikes observed within 30 sec previous to the stimulation was considered as the spontaneous activity In the control group the number of spikes increased slightly with lapse of time, and with a change of Tyrode solution the increase became marked

By the administration of 2.5 γ/ml quinine -drochloride the spontaneous spikes, ap-ximately 500 (100%) before the administra-on decreased in number immediately after the addition of quinine, but 19 min later it then slightly recovered to 300 (60%)

Increase of the spontaneous activity immediately after the change of Tyrode solution was temporary and complete recovery could not be observed.

By quinine administration at concentration of 5 γ/ml spikes decreased from 450 (100%) to 45 (10%) recovery by change of Tyrode solu tion being only 90 (20%)

By the administration of quinine hydrochloride of 10 γ/ml and 20 γ/ml the inhibition of spontaneous activity was marked and change of Tyrode solution after 43 min resulted in no recovery

DISCUSSION

Quinine has long been used for the treatment of malaria and it is also well known for years that hardness of hearing, tinnitus, vertigo, blurring of vision and photophobia may occur during and after quinine therapy and that the administration of a large quantity may possibly cause delirium, dyspnea or even death of the patient.

Concerning the ototoxicity of this substance Falbe Hansen (1941) has reported this to be a conductive deafness, whereas Forbes (1943) and Rüedi *et al* (1952) and many others considered it to be a perceptive deafness.

Wittmaack (1936) considered the change of the spiral ganglion, especially of the Nissl's bodies, and the hypotonic degeneration of the cochlear receptors as changes produced by quinine This hypothesis was supported by Mygind s (1945) so called endolymphatic compression theory But from the fact that this hypotonic degeneration, which was ascribed to collapse of the Reissner's membrane, has often been found both in animals fixed with intravital and postmortem perfusion, which is regarded as an artifact by Fernández (1958).

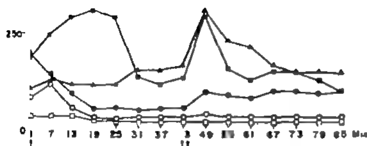


Fig. 3. Action of quinine hydrochloride on the spontaneous activity of the ampullar receptors.

Imp: counted spikes within 30 sec. Addition (↑) and removal (↑↑) of the quinine. ▲–▲ control, ■–■ quin. hydrochlor 2.5 γ/ml; ●–● quin. hydrochlor 5 γ/ml, ○–○ quin. hydrochlor 10 γ/ml; □–□ quin. hydrochlor 20 γ/ml.

On the other hand Covell (1938) reported on the degeneration in the stria vascularis, the outer hair cells and the primary cochlear neuron, this being supported by Rüedi *et al.* (1952).

Wittmaack (1936) states that the ototoxicity of quinine is hypotonic degeneration of the cochlear and Covell (1938) proposed that it is the degeneration of the hair cells, stria vascularis and the primary cochlear neuron. Recently Hennebert & Fernández (1959) made a further investigation. They injected quinine solution into the middle ear space of guinea pigs, and observed the abnormality of postural and post-rotatory nystagmus and signes usually seen after unilateral labyrinthectomy. In the vestibular receptors distruction was histologically proved. He also examined the influence of quinine on the action potential (AP) and cochlear potential (CM) and observed a remarkable decrease in electric potentials. In the histology of these animals destruction of the outer hair cells, limbus and stria vascularis was revealed, which was more marked in the neighboring portion to the round window. Corti's apparatus was necrotic. In animals suffering from chronic intoxication with quinine the cochlear response was extinguished or at least disordered. The conductive mechanism was also destroyed extensively. Histopathologically the outer hair cell was strongly damaged in the basal turn stria vascularis and Corti's organ were reported to have disappeared in places.

The author investigated the change of the electric response after direct action of quinine hydrochloride on the isolated posterior semicircular canal receptor of frogs. At concentrations less than 1 γ/ml of quinine hydrochloride in Tyrode solution the change of response was insignificant.

But at 2.5 γ/ml concentration of quinine strong influence on the response was observed, the number of spikes after quinine administration descended to some 70% of that which was found prior to administration. Change of Tyrode solution to wash off the drug after 43 min operation did not provide sufficient recovery.

By 5.0 γ/ml quinine hydrochloride the number of spikes decreased to some 40% of initial activity with a fall of potentials. After washing off the medicament the discharge recovered up to 70% of the initial activity.

At 10 γ/ml and 20 γ/ml concentrations response in the form of spikes diminished to 28% and 20% respectively and recovery following exchange of Tyrode solution was as insufficient as 50%.

At each concentration the inhibition of both the number of spikes and amplitude of potentials was remarkable immediately after quinine administration, and became still more marked

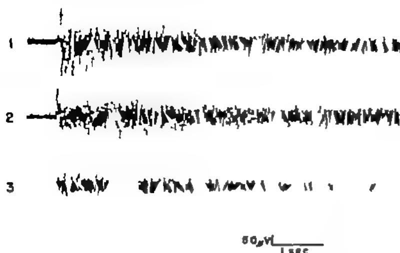


Fig 4 Response to stimulation of the ampullar receptors of the isolated posterior semicircular canal: (1) before adding quin. hydrochloride (2) 19 after administration of quin. hydrochlor 2.5 γ/ml; (3) quin. hydrochlor 20 γ/ml.

with the course of time. Recovery of the response by washing with Tyrode solution ran rather slowly reaching its peak at 24 min after washing, followed by a slight decrease. This fact may be due to the solubility of quinine or to its strong affinity to the surface membrane of the semicircular canal receptors.

Observation of spontaneous activity showed that with higher concentration of quinine the spontaneous spikes became less numerous and recovery to the initial activity by washing with streptomyces antibiotics was not seen. With Kanamycin, for example spontaneous activity showed marked increase immediately after washing off the medicament (Harada, 1966 7)

Hennebert & Fernández (1959) dropped quinine solution on the round window to investigate changes of CM and AP He reported that cochlear response rapidly fell within several minutes and the amplitude of CM diminished to 50% after 12 min

In the present study a 2.5 γ/ml concentration of quinine was enough to affect the activity of the semicircular canal receptors, and with 20 γ/ml a very strong inhibition of the potentials and a decrease in the number of spikes was observed. Recovery after washing with Tyrode solution was incomplete in preparations with higher concentrations. Further longer action made the inhibition irreversible.

Whether this inhibitory effect on the electric activity is due to direct action on the receptor cells interfering with respiratory metabolism, or due to some influence on the membrane potential is still unknown. However Hennebert & Fernández (1959) in his acute experiment, lesions by quinine injection into the middle ear space, reported a degeneration in the Corti's apparatus and the vestibular sensory cells. From the present findings, it might be considered probable that quinine has some noxious action on the sensory cells of the semicircular canal

ZUSAMMENFASSUNG

Die Wirkung verschiedener Quininhydrochloride auf die ampullaren Rezeptoren des isolierten hinteren Bogenganges des Frosches wurde an Hand von elektrophysiologischen Methoden beobachtet. In der mit 2.5γ/ml Quininhydrochloride hinzugefügten tyrodeschen Lösung unterdruckten sich schnell sowohl die Reaktivität auf Reizung der ampullaren Rezeptoren als die spontane Aktivität, die bei 20 γ/ml Konzentration fast völlig gehemmt wurden. Nach Abwaschen des Quinin liess sich die Reaktivitat gegen Stimulation der ampullaren Rezeptoren sowie spontane Aktivität ein wenig wiederherstellen Die Bedeutung dieser Ergebnisse wird diskutiert.

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HABITUATION OF NYSTAGMUS AND SENSATION OF MOTION AFTER ROTATION

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The habituation effect of repeated rotatory stimulation on normal young adults was studied. A total of 70 identical rotatory stimulations were applied and the postrotatory nystagmus was registered by the photoelectric (PENG) technique. Keeping the eyes open in darkness, the frequency of the nystagmus was counted and no response decline was observed. On the contrary, some increase in the intensity of the postrotatory nystagmus was found. The perception of the sensation of motion following the sudden stop of a 180°/sec angular velocity was also studied. Great variability was encountered but no consistent response decline occurred. The duration of the post-rotatory nystagmus showed similar characteristics, i.e., variable responses but no signs of habituation.

The present study will be concerned primarily with the nystagmus response as induced by means of a rotatory acceleratory and deceleratory stimulation as well as the sensation of turning experienced following a complete deceleration of the rotatory stimulus.

Hundreds of studies have concerned themselves with the variables of the rotation-induced nystagmus. One of these variables is that of habituation. The term "habituation" as it refers to the nystagmus reaction was first introduced by Abels (1906). Henriksson *et al* (1961) utilized the term to indicate the phenomenon of progressive reduction of the nystagmus response to either repetitive rotatory or caloric stimuli. Even more specifically, Barber & Wright (1967) have defined habituation as "a response decline due to repeated semicircular canal stimulation, caloric or rotational". The term "response decline" to describe the process of habituation was originated by Hallpike & Hood (1953) in an effort to eliminate certain difficulties of terminology concerning the effects of fatigue and adaptation and to allow those authors and other researchers to speak of the habituation process without specifying which of the two phenomena (fatigue or adaptation) was the causal factor.

Habituation as it concerns rotation-induced nystagmus

According to Griffith (1920), the method of rotation to elicit a nystagmic reaction was first used by Purkinje as early as 1827, and almost every investigator since his time has used it in one form or another. Habituation of the nystagmus response to these rotatory stimuli has been observed in humans by many researchers. However, the procedures for measuring and recording their objective responses have varied widely, making it difficult to determine whether their specific experimental conditions could have caused the apparent variance in their reported results. For example:

1. Dodge (1921, 1923) utilized a closed-eye technique to record the nystagmus and, by means of a mirror recorder, recorded photographically the eye movements that were hidden under the closed lids. Results demonstrated that the amplitude of post-rotation nystagmus (after-nystagmus) decreased from day to day throughout the experiment. There

was also a tendency for the amplitude to decrease from record to record within each experimental day"

2. Griffith (1920) utilized an open-eye technique with an experimenter counting and recording the number of nystagmus responses. An acceleration to full speed of 180°/sec was obtained in one second. After a gradual stop the post-rotatory nystagmus responses were recorded and led to the following conclusions: "As turning is repeated from day to day the duration of the after-nystagmus, the number of ocular movements made, and the duration of the apparent movement rapidly decrease. The major part of this decrease occurs within the first few days. The decrease takes place not only from day to day but also within a period of ten trials on any single day. The amplitude of the ocular movements and the number of movements made per second also decrease as repetitions increase." It was also found that "the time [duration] of nystagmus changes with the speed of rotation and with the number of revolutions and that it is increased when the chair is abruptly halted"

3. Brand (1968), utilizing a closed-eye technique for recording nystagmus responses, exposed his subjects to various degrees of angular acceleration from a minimum of 7.5°/sec to a maximum of 60°/sec in varying orders. Using a measure of the angular velocity of the slow phase of the post-rotational nystagmus responses, he was able to demonstrate that habituation did occur on the first three of nine consecutive days in much the same way as the subjective turning sensation decreases.

4. Torok (1969) utilized a standardized photoelectronystagmographic technique (PENG; Nykiel & Torok 1963) with goggles over open eyes in darkness. Weaker (45°/sec end velocity followed by a sudden stop) and stronger (90°/sec followed by a sudden stop) rotatory stimulations were administered. A comparison of two parameters of the nystagmus responses, nystagmus frequency count and slow phase velocity, was carried out. It was considered that the former is possibly the better parameter of nystagmus intensity. Of importance to the present paper was the fact that the results indicated that no sign of habituation existed. Moreover, an increase in intensity with repetition was seen in two of the eight subjects.

The above few examples illustrate the point that a nystagmus response decline does appear to occur under some experimental conditions while not under others. Possible important variables appear to be (1) the condition of the eyes, whether open and fixated in light, open in darkness, or closed during the recording of the response, and (2) the method of evaluating the nystagmus response (frequency count, slow phase velocity, nystagmus duration, or amplitude of the nystagmus). Many other variables appear to exist such as stimulation strength obtained during the course of each trial, the state of alertness or relaxation of the subject, direction of rotation, time interval between consecutive test, environmental conditions (extravestibular stimulations such as noise and light).

One of the purposes of the present study is to demonstrate that under certain controlled conditions, it is possible to perform several consecutive trials utilizing rotatory stimuli without producing the phenomenon of "habituation" or "response decline". In clinical situations it may be necessary to repeat certain test procedures. On the other hand, it has been mentioned (Guedry 1965) that the effect of response decline may last for periods of days or weeks. Therefore, it would be of importance to determine whether or not under certain test conditions a response decline may be eliminated in spite of repetitions of stimulation.

Habituation as it concerns rotation-induced subjective sensation of movement

Many researchers (Griffith, 1920; van Egmond *et al.*, 1948; Hallpike & Hood, 1953; Henriksson *et al.* 1961; Aschan *et al.* 1956; Forssman, 1963; to name a few) have studied the perception of movement which occurs during and after rotatory, caloric or other stimuli.

This phenomenon is often described as dizziness, sensation of spinning, sensation of movement, etc Concomitant with the sensation of dizziness elicited by vestibular stimulation, autonomic nervous reactions frequently occur These reactions may take the form of nausea, vomiting, perspiration, flushed face or facial pallor etc. These reactions have been reported to show a response decline (Loch & Haines, 1946) but this should not be equated with the concomitant perception of motion which is of interest in the present study

In the present paper interest in the response decline of the perception of motion will center upon the problem of its occurrence with repeated rotatory stimulations—especially as it occurs in conjunction with the deceleratory phase of rotatory stimulus. In his study of the "Post rotational turning sensation" Brand (1968) pointed out that there was clear evidence of a response decline probably attributable to habituation processes which are akin to the 'tolerance which develops on repeated exposure to other forms of unfamiliar labyrinthine stimulation" Guedry (1965) stated that the major response decline seemed to have been restricted to the unnatural anti-synergic sensory input occasioned by head movements during the whole-body rotation" nd that (for cats) it may be specific to the ection of response repeatedly elicited during bituation

Benson *et al* (1966) stated that their experiment demonstrated unequivocally that the duration of the reported after-sensation evoked by an impulsive angular stimulus was influenced by the instructions received by the subjects" Also mentioned was the fact that strong instructions could increase the duration of the after sensation, thus eliminat ing the effects of a response decline while weak" instruction allows habituation to oc cur Although there was no proof that intensity of arousal was enhanced in the strong instruc tion condition, the authors inferred that this provided a definite possibility for the presence or absence of habituation of after-sensation

This condition of relaxed mental alertness was also noted by Torok (1969) in reference to the elimination of habituation of the nystagmus response He referred to this state as one of relaxed attention" Torok indicated that relaxed attention could be brought about simply by instructing the subject to "keep his eyes open in the mid-position, and to avoid blinking as much as possible" To prevent the effects of eye fixation from light or other extraneous stimuli, the subject's eyes were kept open in total darkness with the use of the photoelectric nystagmus-pick-up goggles (PENG)

Thus, it will be noted that under certain test conditions, some researchers have found it possible to eliminate the effects of response decline of the perception of motion. Hulk & Jongkees (1948) attempted to furnish information for a normal sensation cupulogram derived from the information gathered from testing 50 normal subjects. They thought that (from a diagnostic standpoint) there is as much quantitative importance to be gleaned from the sensation cupulogram as from the nystagmus cupulogram

In the present study it will be of interest to determine whether under some pre-established test conditions the response decline of the perception of motion might be observed.

In summary the two questions to be answered in this experiment are the following:

1 Under some pre-established test conditions, is it possible to eliminate the effects of response decline in the post rotatory nystagmus response to repeated rotatory stimulations?

2. Under the same test conditions, is it possible to eliminate the effects of response decline in the post rotatory perception of movement?

METHOD

Apparatus and conduct of experiment

Rotatory stimulations were performed on five volunteers. Only counterclockwise rotations have been used. Angular acceleration at a rate

of 2.5 /sec² to a maximum of 180 /sec was applied. This constant velocity of 180 /sec was maintained until all acceleratory nystagmus had subsided completely as indicated by the nystagmogram. The chair was stopped instantly through an electromagnetic braking mechanism causing the constant velocity speed to drop from 180 /sec to a complete stop in less than one second. Nystagmus was recorded by means of the photoelectric technique (PENG) described by Torok *et al* (1951) and Nykiel & Torok (1963).

The subject was seated comfortably and the following instructions were given. In a few moments, the chair in which you are seated will begin to turn. However this movement will probably not be rapid enough to cause any apparent sensation. It will turn for approximately one minute, followed by a sudden stop. You will be warned just prior to stopping the chair. It is important that during this entire procedure you keep your eyes straight forward and do not blink. After the chair has stopped, you may have the sensation of moving in the opposite direction. Immediately at the beginning of the sensation, you must say NOW". Just as soon as the moving sensation disappears, you will indicate it by saying "STOP." (If the instructions were not clear enough, they were repeated until the subject indicated he understood.)

Following the instructions, the photoelectric goggle was placed over the subjects eyes, and an open adapter plate over one eye allowed for calibration adjustments to be accomplished until a 10° eye deflection to left or right corresponded to a 10 mm pen deflection on the recording graph paper. The subject's head was then placed in an approximate 30 forward position (and held in position against a headrest) to insure optimal stimulation to the semicircular canals. The adapter plate was then closed and all room lights turned off.

During all phases of the test, records of the nystagmus responses were made with standard Dynograph recording equipment which allows the response to be instantly viewed. A technique for counting nystagmus in five second intervals was utilized and has been described fully by Torok (1948, 1966) and by Torok & Derbyshire (1969). In accordance with Torok's findings (1968) the frequency of the fast phase of the nystagmus response was used to establish nystagmus intensity. Between two successive trials, a five minute rest interval was allowed. During this period, room lights were turned on, the adapter plate opened and the subject asked to relax his eyes by closing or opening them as desired but the goggles were not removed.

During each of the four test days extending over a two week period, five trials separated by five minute intervals were administered making a total of twenty trials for each subject.

Perception of movement was recorded by the examiner marking the beginning and end of the sensation directly on the graph paper as indicated by the subject's NOW" and "STOP" responses as mentioned above.

Subjects

The subjects consisted of five volunteers, four men and one woman, aged 20 to 36 years. None of the subjects had any history of neuro-otological or hearing impairment. The subjects were graduate students or medical students. No drug administration for at least 24 hours or cigarette smoking for at least one hour prior to the test administration was allowed.

RESULTS

Habituation as it concerns rotation-induced nystagmus

This preliminary study was designed primarily to determine whether it would be possible under certain controlled clinical test conditions to prevent response decline in both the post rotatory nystagmus and after-sensation of movement. The design of the stimulation series as described above allowed the experimenter to look for any effects of habituation within individual tests on a single day as well as the same effect over an extended period of time

Torok (1969) stated that it is possible under certain test conditions and without the aid of especially designed arousal techniques such as mental arithmetics to keep the patient in a relaxed condition of mental alertness These test conditions are met in the PENG technique which employs the use of special goggles with a photoelectric pick-up unit. The technique allows the patient to keep his eyes in a relaxed, but wide-open state while maintaining darkness to prevent fixation or other intervening variables encountered in a light environment. It was observed that not only was there no sign of habituation in the nystagmus response with repeated stimulation under these conditions, but two of eight subjects actually demonstrated an increase in nystagmus intensity with repeated stimulations.

Some of the results of the present study are illustrated in Fig. 1 which is an averaged post rotatory nystagmus frequency count for the first and fifth trials for all five subjects over the four testing sessions. This figure demonstrates that for five subjects with normal hearing and vestibular functions and utilizing the test conditions referred to above, habituation does not occur with repeated stimulation. Also indicated in Fig. 1 is the fact that an intensity increase from Trial 1 to Trial 5 occurs roughout the entire duration of the post atory nystagmus response Although not own in the figure this enhancement of re-



Fig 1 A eraged post-rotatory nystagmus frequency count for the first and last of 5 consecutive trials conducted during four testing sessions on 5 subjects. Means for the 5 subjects are shown for each 5-second interval. Standard errors are shown for the first two 5-second intervals only

Table 1 *Contingency table demonstrating the actual number of times the nystagmus frequency count of Trial 1 was greater than that of Trial 5 and the probability of such an event*

Trial no	No. of actual response occurrences	Probability of response
1	19	42.5
5	66	42.5

sponse phenomenon was observed to exist between the trials of all subjects during all of the test days in the series. Also evident in the figure is the orderly fashion in which the intensity increase has occurred throughout the entire duration of the response curve.

Accepting the hypothesis generated by past work concerning habituation, the frequency count of Trial 1 will always be greater than Trial 5 The Sign Test was applied to a sample of pairs of observations (Trial 1 versus Trial 5) Table 1 is a contingency table made up of these sample pairs of observations. It was found that only 19 times out of 85 was the nystagmus frequency value of Trial 1 greater than Trial 5 Utilizing the values in the contingency table, χ^2 was worked out by means of the following formula.

$$\chi^2 = \frac{2m \pm 1}{\sqrt{N}} \quad 1 \lambda$$

This data yielded a χ^2 value of 7 88 and converted to a Z score of 2.81 Entering a normal distribution curve with this Z value yielded an alpha of 0 003 Therefore, the distribution found could only occur 0.3 percent of the time by chance. Now the problem is how to explain post rotatory enhancement of response with repetition of the stimulus.

DISCUSSION

In order to explain the conditions necessary for the elicitation of response enhancement with repeated stimulation, some of the following conditions will need to be examined.

1 *Eye condition* Will response enhance-

ment occur under all open and closed eye conditions? Brand (1968) demonstrated that habituation occurs when the closed-eye technique is used in conjunction with the ENG technique of recording the post rotatory nystagmus response. Griffith (1920) demonstrated the presence of habituation of the nystagmus response with an open-eye technique in a lighted environment with an experimentor counting and recording the number of nystagmus responses. Torok (1969) has indicated that the effects of habituation of the nystagmus response can be eliminated by the use of PENG (open eyes in darkness). Therefore, since the PENG technique was utilized in the present study it seems essential to determine if enhancement of nystagmus response occurs under other eye conditions as well.

2. *Instructional technique* Is the method of strong instruction necessary for eliciting enhancement of response? Benson *et al.* (1966) were able to show that the decay of post-rotatory nystagmus was prolonged by the effect of strong instructions. In the present study the instructions have received additional strength, perhaps, by requesting the subject to observe and indicate the duration of the after sensation of movement. Torok (1969) has indicated that simple instructions such as to keep the eyes open, straight ahead and without blinking for the duration of the test lend enough strength to prevent habituation of nystagmus response. Therefore, during future experiments it will be necessary to determine whether this simple instructional technique will suffice to elicit response enhancement with repeated stimulation.

3 *Type of stimulus* Does response enhancement occur only under the stimulus conditions of the present study: that is, an acceleration speed of 2.5 /sec² in a counterclockwise direction to a constant angular velocity of 180 /sec followed by an abrupt deceleration? Brand (1968) has demonstrated the presence of habituation in conjunction with several accelleratory speeds. In the Benson *et al* study (1966) the direction of rotation (clockwise or counterclockwise) apparently had no effect on the post rotational nystagmus response as calculated by a measure of angular velocity of the slow phase allowing the experimentors to combine the values for rotation to right and left. Henriksson *et al.* (1961) have demonstrated the effects of habituation on the calorically induced nystagmus response. Thus, it will be important to demonstrate the presence or absence of enhancement of response under some of these varied experimental conditions using the PENG technique.

4 *Data evaluation* Enhancement of response has been established with nystagmus frequency used as a measure of response intensity. *Hood & Pfaltz (1954)* have noted the absence of response decline in man following caloric stimulation when the duration or frequency of nystagmus were used as measures of response intensity. Brand (1968) utilized the measure of slow phase velocity to demonstrate the presence of habituation of the post-rotatory nystagmus response. Although Torok (1969) has demonstrated the effectiveness of the frequency count as a measure of the intensity of the nystagmus response, a future study should attempt to determine whether or not the angular velocity of the slow phase will demonstrate the same type of response enhancement that a count of nystagmus frequency did in the present study.

5 *Pre-established mental set.* The enhancement of the postrotatory nystagmus response could be dependent upon the mental set with which the subject first enters the test environment.

During the present experiment, it is possible that at least two variables were contributing factors for the pre-establishment of a mental set on the part of the subjects as they first entered the test environment; (*a*) previous experiences they may have had in which dizziness and its frequent concommitant autonomic nervous reactions following some type of intense rotational stimulation (skating, carnival rides, etc) and (*b*) a concomitant increase in the mean number of nystagmus beats which

Table 2. *Illustration of the changes in means and standard deviations of the nystagmus frequency response taking place during the first two 5-second intervals over 4 test sessions*

The figures include all 5 trials for all 5 subjects

Test no.	1st 5-second interval Mean	s.D.	2nd 5-second interval Mean	s.D.
1	15.04	3.24	12.44	2.55
2	15 64	2.61	14.08	2.10
3	18.64	2.40	13.84	2.39
4	19.32	_.18	16.00	1.87

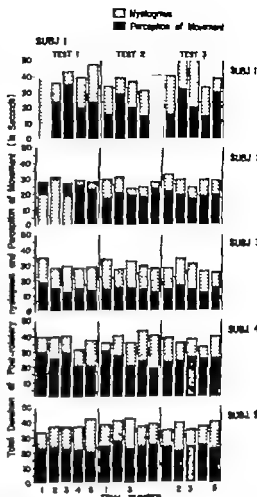


Fig 2 Diagramatic representation of duration of post-rotatory nystagmus and post-rotatory perception of movement following repeated rotatory stimuli at 180° per second in a counterclockwise direction.

could be recorded with each subsequent test. Table 2 illustrates this phenomenon with the means and standard deviations for the first two five-second intervals for all subjects Although the differences from test to test were not always significant, a definite and consistent trend does appear The same type of trend could be seen for each subject within each test session

Further evidence in support of the possibility of a pre-established mental set comes from consistent remarks made by all subjects except Subject 1 These remarks were to the effect that whereas they had usually approached the initial trial in each test with some anxiety by the fifth trial they were somewhat d, but actually enjoying their experience. s anxiety over the initial trial was never strong during the last two tests and had subsided almost completely as the subjects approached the final test session. Griffith (1920) received similar comments from some of his subjects in his study on the effects of dizziness. Thus, it seems plausible that the subject may be entering the test situation with a pre established inhibitory set which may effect the nystagmus response Moreover repeated stimulation without apparent adverse effects may serve to relieve the anxiety and thus, allow for the recording of nystagmus responses like those seen in the response enhancement noted in the present study However much more evidence is needed to support such a theory

Habituation as it concerns rotation-induced subjective sensation of movement

In this study one of the problems was to attempt to establish whether or not under certain test conditions it would be possible to eliminate the effects of a response decline in the post rotatory perception of movement with repeated stimulations The idea was that a stable response might have importance from a clinical standpoint.

Several investigators, as already mentioned, noticed a response decline of perception of movement under certain clinical test conditions. Others have been able to prevent this same decline with other clinical techniques. In

Table 3 *Mean (20 trials) duration of post-rotatory nystagmus and perception of movement responses for 5 subjects*

Stimulation in all cases was an instantaneous stop from constant velocity of 180°/sec counterclockwise rotation

Subject no.	Duration of post rotatory nystagmus (sec)		Duration of post rotatory perception of movement (sec)	
	Mean	S.D.	Mean	S.D.
1	39.55	5 19	23.30	8.62
2	27 10	3.51	23.50	4 16
3	29.65	2.98	14.70	1.56
4	36.90	3.08	22.85	3.80
5	37 15	2.56	23.75	2.02

our study the beginning and ending of the subjective responses were recorded directly on the nystagmogram. Thus, it was possible to look for response decline only through the durational aspects of the reported perception of movement.

Fig. 2 is a diagramatic representation of the durations (in seconds) of both post-rotatory nystagmus and perception of movement responses following repeated rotatory stimuli at 180 /sec in a counterclockwise direction. The responses of all five subjects are reported by trial number In this representation, it should be possible to observe the effects of any response decline both within tests and across tests.

It may be noted that there is much variation for both the nystagmus and perception of movement durations for Subject 1 However there is no systematic response decline for either response. The histograms for Subjects 2, 3 4 and 5 show much less variability for either type of response and definitely no systematic response decline. In almost every case the duration of the post-rotation nystagmus response was considerably longer than the duration of the perception of movement. The only cases in which this condition did not exist were for Trial 1 for Subject 1 and in several instances for Subject 2. However the reasons for this reversal were probably not the same for the two subjects. Subject 1 reported that he was not sure of what he was to look for and after Trial 1 it was necessary that he be reinstructed. In several instances where this reversal occurred for Subject 2, the duration of the two responses was very close, and the reversal might be accounted for on the basis of chance variation. The mean duration of the nystagmus response for onehundred trials (all trials for all subjects) was 34 07 sec with a standard deviation of 5 98 The mean perception of movement duration for the same one hundred trials was 21 62 sec with a standard deviation of 5 81

Table 3 represents the mean durations of the post-rotatory nystagmus and perception of movement responses and their standard deviations for all five subjects. According to an F ratio performed on the variances for the nystagmus responses, the results of Subject 1 are significantly different ($P = 0\ 05$) from those of the other four subjects. Of interest here is the fact that Subject 1 was the only subject that appeared to suffer from the effects of the rotational experience and never felt relaxed throughout the entire test series as evidenced by his continuous complaints of slight dizziness and nausea, etc. This fact may hold implications for the presence of a pre-established psychological set mentioned above Moreover although the mean durations for the other four subjects are quite variable from subject to subject, the within subject variance from trial to trial and test to test is very small and not significant ($P = 0\ 05$) These facts demonstrate that although the mean duration of the post-rotatory nystagmus response is liable to vary considerably between subjects, this type of objective measure provides similar information from each person.

The F-ratio scores for the perception of movement response indicate that there are significant differences ($P = 0\ 05$) between almost all subjects although the intra-subject variability did not appear to be so great. The indications here are that each subject is responding to his own concept of perception of movement. Since this concept varies substantially between

subjects, it does not appear to be an adequate measure for diagnostic use as has been recognized repeatedly by clinicians.

DISCUSSION

The instability of the durational aspects of the nystagmus response has been recognized for a long time (Torok, 1948 1962) Buys, as early as *1920* called attention to the frequent discrepancy between the nystagmus duration and the intensity of the post rotatory nystag mus response. A similar problem has often been cited in reference to the instability of the perception of movement response and its sus ceptibility to response decline

The implications of the present research are that by utilizing the PENG technique in conjunction with the specific conditions mentioned above, a response decline does not occur for the durational aspect for either post rotatory nystagmus or the perception of movement with repeated stimulation. As far as the reliability of the duration of these two responses is concerned, neither of them provide the type of information and stability necessary to suggest their incorporation into the clinical diagnostic schemata.

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ZUSAMMENFASSUNG

Der Habituationseffekt nach wiederholtem Drehreiz wurde an jüngeren Erwachsenen studiert. Nach 20 Drehungen wurde der postrotatorische Nystagmus durch die photoelektrische Methode (PENG) registriert. Bei im Dunkeln geöffneten Augen wurde die Nystagmusfrequenz gemessen. Eine Intensitätsverminderung wurde nicht beobachtet. Im Gegenteil wurde eine leichte Intensitätszunahme *festgestellt. Die* Wahrnehmung des Drehgefühls, das auf das plötzliche Anhalten nach Drehgeschwindigkeit von 180° pro Sekunde folgt, wurde auch studiert. Grosse Reaktionsunterschiede wurden regelmässig gefunden, ber keine Intensitätsverminderung. Die Dauer des postrotatorischen Nystagmus zeigte ein ähnliches Verhalten, nämlich unterschiedliche Reaktionen, ber keine Zeichen von Habituation.

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LABYRINTHINE DEFECTS AS SHOWN BY ATAXIA AND CALORIC TESTS

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Most of the individuals with labyrinthine defects severe enough to produce ataxia test performance scores within the lowest 5% of the normal distributions also showed threshold caloric test responses which fell within the same range Similarly almost as many individuals with decreased threshold caloric test responses also showed poor ataxia test performance scores. While vestibular influences governing threshold caloric test responses are far more specific than those affecting results on the ataxia tests, the high agreement shown between results of each in groups of individuals with various loss or disturbance of labyrinthine function implies a common vestibular effect to a considerable degree

As part of our study of the problems of man s gravitoinertial force environment in space flight, particular attention has been given to testing the vestibular organs to determine their role in man s ability to withstand such an environment. A threshold caloric test (McLeod & Meek 1962) and an ataxia test battery (Graybiel & Fregly 1966 Fregly & Graybiel, 1968) were introduced recently as part of the testing procedure at this laboratory They serve as rapid, reliable screening tests for selecting individuals without known labyrinthine defects from those who have such defects, and provide a means for comprehensively studying their vestibular apparatus.

Slight, even moderate, degrees of vestibular ataxia may go unrecognized in persons who either do not challenge their capabilities in this regard or misinterpret their handicap as poor athletic ability" Moreover ataxia as well as a loss of semicircular canal function may go undetected during routine medical evaluations because of insufficiently precise measurements. Our findings with the threshold caloric test and the quantitative ataxia test battery have led us to suspect abnormal scores on both if either one is abnormal. The purpose of this report is to demonstrate their diagnostic value independently and jointly based on an analysis of the scores obtained on 365 individuals classified either as normal subjects or as persons with various diseases and disorders of the inner ear

PROCEDURE

Subjects

The vestibular normal group consisted of 240 men, in the age range of 17 to 63 years, who had been tested by the threshold caloric and by the ataxia test battery The group included student military aviators, volunteer Navy enlisted research subjects medical students, Navy deep sea divers, individuals with varied experience on centrifuges, and military and civilian technical and scientific personnel. All were in good health and free of any known vestibular disturbance, and had had benefit of recent medical and audiometric evaluations.

The group of patients with vertigo included men and women ($N = 76$), with an age range of 18 to 71 years, who were referred from mili-

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tary and civilian physicians because of vertigo as a major symptom or complaint. The great majority of these referrals presented a diagnostic problem some had received a tentative diagnosis, while the symptoms of others could not be diagnosed. Diagnoses of the remaining few individuals represented the following classifications. vestibular neuronitis, typical and atypical Menière's syndrome, postural vertigo, positional *nystagmus*, *labyrinthine artery* occlusion, post-traumatic pseudobulbar palsy acoustic trauma, longstanding deafness, orthostatic lightheadedness, cerebral vascular insufficiency and psychogenic disorder The health and physical status of these patients were adequate for undergoing testing.

The group of bilateral labyrinthine-defective (BL D) subjects consisted of 26 men, 18 to 48 years of age, in whom meningitis or mastoiditis had developed early in life. Ten of these subjects have participated frequently in the vestibular research program at this laboratory (Graybiel, 1964 and 1966) the other 16 subjects with highly similar afflictions had been screened recently from a larger population of deaf students and faculty at Gallaudet College for the purpose of their participating in vestibular research. The health and physical status of these individuals ranged from good to excellent.

Eleven individuals constituted the group of unilateral labyrinthine-defectives (UL-Ds) and included men and women, in the age range of 33 to 58 years, who were treated surgically for acoustic neuroma (House, 1964) one-half year to three years prior to threshold caloric and ataxia testing. Their health and physical status were adequate for undergoing testing.

The group with Menière's syndrome, treated with streptomycin sulfate ten years previously (Graybiel *et al.* 1967) was composed of three men, 42, 50 and 56 years of age, and one woman, 49 years of age. Their health and physical status were adequate for testing.

Another group consisted of five men, 21 to 34 years of age, in whom total or near total deafness had occurred as a result of head injury and who were screened from a larger population of deaf students and faculty at Gallaudet College. All were in excellent health and good to excellent physical condition.

The group of congenitally deaf subjects consisted of two men, 19 and 20 years of age, and one woman, 19 years of age As established from available health records, questionnaires, and interviews, all of this group had been totally deaf since birth or before their first birthday The etiology was not known. Their health and physical condition were good to excellent.

APPARATUS AND METHOD

Threshold caloric test. The apparatus and procedures were highly similar or identical to those described previously by McLeod & Meek (1962)

Rail and floor ataxia test batteries The seven performance test items are described here only briefly since detailed descriptions have been previously published (Graybiel & Fregly 1966 Fregly & Graybiel, 1968) The tests were administered in the following sequence: (1) Sharpened Romberg (SR), consisting of standing on the floor with eyes closed for 60 sec (2) rail walking and rail standing (3) standing on one (each) leg on the floor with eyes closed for 30 sec (SOLEC R and SOLEC L) (4) walking a 12 foot line on the floor with eyes closed (WALEC) scored as inches of deviation from the line Rail walking and rail standing consisted of (*a*) walking with eyes open (Walk E/O) on a /₄-inch by 8-foot rail, scored as number of steps (maximum of five steps per trial) (*b*) standing with eyes open (Stand E/O) on the /₄-inch rail, scored to the nearest second (maximum of 60 sec per trial) and (*c*) standing with eyes closed (Stand E/C) on a 2 / by 30-inch rail, also scored to the nearest second with a maximum of 60 sec per trial.

RESULTS

The distribution of the threshold caloric responses of the normative" group and of the

group of patients referred because of vertigo are shown in Tables 1 and 2 respectively and the individual threshold caloric test responses in the five other groups of subjects are shown in Table 3

Abnormal" threshold caloric responses are defined arbitrarily as responses having a 5th percentile or lower equivalent (≤34.5 C) in relation to the distribution of responses in the less sensitive ear of the normative group (Table 1) Similarly abnormal ataxia test performance skills are defined arbitrarily as scores having a 5th percentile or lower equivalent in relation to normative standards of performance in various age classifications, as reported elsewhere (Graybiel & Fregly 1966 Fregly & Graybiel, 1968) For example, scores having 5th percentile rankings in a control (standardization) group of 17–42 year-old normal men were 171 (SR) 6 (Walk E/O) 11 (Stand E/O) 15 (Stand E/C) 56 (SOLEC R) 59 (SOLEC L) and 24 (WALEC) and in a control (standardization) group of 18–29-year-old women the corresponding scores were 35 5 11 16 27 36 and 24 on these tests

Table 1 *Frequency distribution of threshold caloric response levels and their percentile equivalents in the control (normative) group of "vestibular normal" Men (N – 240)*

tted line – arbitrary cut-off criterion of "abnormality"

Less sensitive ear			More sensitive ear		
N	Response interval (°C)	Percentile equivalent	N	Response interval (°C)	Percentile equivalent
162	≥36 0	34th–99th	201	≥36.0	17th–99th
20	35.8–35 9	33rd	15	35.8–35 9	16th
14	35.6–35.7	24th	10	35 6–35 7	10th
10	35.4–35.5	18th	7	35 4–35.5	6th
10	35.2 35.3	14th	4	35.2 35.3	3rd
7	35.0–35 1	10th	2	35.0–35 1	1st 2nd
4	34.8–34 9	7th	0	34.8–34 9	
1	34 6–34 7	5th–6th	0	34 6–34 7	
5	33.6–34.5	5th	1	34.4–34.5	1st
2	32.6–33.5	3rd			
1	30.6–32.5	2nd			
4	20.6–30.5	1st 2nd			

Table 2. *Frequency distribution of threshold caloric responses in patients with vertigo (N – 76)*

Dotted line – arbitrary cut-off criterion of "abnormality

N	Age range (yrs)	Mean age (yrs)	Threshold caloric response levels in less sensitive ear (°C)	Threshold caloric response levels in more sensitive ear (°C)
34	21–69	38.4	≥36.0	36 0–36.8
8	27–61	42.3	35.8–35 9	35.8–36.6
6	38–50	44.8	35.6–35 7	35.8–36.6
11	20–68	39.0	35 4–35.5	35.4–36.4
2	68–72	70.0	35.2 35.3	35.6–35.8
4	41–69	53.5	35.0–35 1	35.8–36 4
0			34.8–34.9	
0			34.6–34.7	
3	22 50	32.7	33.6–34.5	34.0–36.3
2	20–53	41.5	32.6–33.5	34.2 36.0
1	22	22.0	30.6–32.5	32.4
2	40–42	41.0	20.6–30.5	32.6–36.2
2	41–58	49.5	11.6–20.5	18.4–36.2
1	47	47.0	10.6–11.5	35.8

Group differences in abnormal responses to tests

As shown in Table 4 the threshold caloric response of all of the BL Ds, Menière s patients, and UL-Ds were abnormal as defined by the arbitrary cut-off criterion, 60% and 67% of the head injury deaf and congenitally deaf individuals, respectively and 14% of the patients with vertigo also had abnormal threshold caloric responses. The group differences in the frequency of abnormal ataxia test scores very nearly paralleled those in caloric responses. Generally the greater the vestibular loss, as shown by decreased response to caloric stimulation in the less sensitive ear the greater was the likelihood that such loss would be reflected also by impaired ataxia test performance Of particular interest was the finding that individuals with various loss of labyrinthine function were more often identified as being abnormal by their floor ataxia test battery scores than by their rail ataxia test battery scores.

Between-test predictability

The percentage of subjects in each group with abnormal ataxia test scores who also had abnormal threshold caloric test responses, and

Table 3. *Threshold caloric test responses of various groups*

BL-D = Bilateral labyrinthine-defective. UL-D = Unilateral labyrinthine-defective (surgically treated for acoustic neuroma). MP = Menière's patients treated with streptomycin sulfate. HID = Head injury deaf. CD = Congenitally deaf

Group	Subject	Threshold caloric responses in °C: Right ear	Left ear	Group	Subject	Threshold caloric responses in °C: Right ear	Left ear
BL-D	1	Neg. at 10.0	Neg. at 10.0	BL-D	26	Neg. at 10.8	Neg. t 9
BL-D	2	No response	No response	UL-D	27[a]	35.2	Neg. t
BL-D	3	10.0	10.0	UL-D	28	36.6	Neg. at 5
BL-D	4	Neg. at 2.8	Neg. at 2.8	UL D	29	34.0	Neg. t 9 (
BL-D	5	Neg. at 3.0	Neg. t 3.8	UL-D	30	35.8	Neg. a. 10
BL-D	6	10.0	No response	UL-D	31	Neg. at 9.6[b]	36.0
BL-D	7	Neg. at 2.8	7.9	UL-D	32	36 0	Neg. at 9 6
BL-D	8	Neg. at 2.6	Neg. at 2.6	UL-D	33[a]	Neg. at 10 0[b]	35 4
BL-D	9	9.8	NIL	UL-D	34[a]	Neg. at 10.0[b]	36.2
BL-D	10	10.0	11.0	UL-D	35[a]	Neg. at 10.0[b]	36.0
BL-D	11	2.8	2.8	UL D	36[a]	Neg. at 10.0[b]	36.2
BL-D	12	Neg. at 10.0	Neg. at 10.2	UL D	37[a]	35 4	Neg. at 10.4
BL-D	13	24 4	14.0	MP	38	31.5	Neg. at 12.0
BL-D	14	Neg. at 9.6	Neg. at 9.6	MP	39[a]	Neg. at 12.0	25.0
BL-D	15	Neg. at 9.6	Neg. at 9.8	MP	40	Neg. at 12.0	Neg. at 12.0
BL-D	16	10.0	Neg. at 9.6	MP	41	30.6	Neg. at 12.0
BL-D	17	Neg. at 9.6	Neg. at 9.8	HID	42	32.0	32.0
BL-D	18	Neg. at 9.8	10.2	HID	43	34.2	33.0
BL-D	19	Neg. at 9.8	Neg. at 9.8	HID	44[a]	35.6	35.6
BL-D	20	Neg. at 9 8	Neg. at 10.0	HID	45	35 4	35 4
BL D	21	Neg. at 10.0	Neg. at 10.0	HID	46	Neg. at 11.0	Neg. at 11.0
BL-D	22	Neg. at 9.8	Neg. at 10.2	CD	47	Neg. at 9 4	Neg. at 10.4
BL-D	[illegible]	Neg. at 9.6	Neg. at 10.0	CD	48	30.0	35.0
BL-D	24	Neg. at 10.2	Neg. at 10.8	CD	49[a]	35.2	34.8
BL-D	25	Neg. at 9.6	Neg. at 10.2				

[a] Women. [b] Surgically treated ear

Table 4. *Group differences in the percentage of abnormal threshold caloric responses in the less sensitive ear and of abnormal rail and floor ataxia test battery scores*

Subject groups	N	Threshold caloric test (%)	Rail ataxia test battery: Walk E/O (%)	Stand E/O (%)	Stand E/C ( )	Floor ataxia test battery: SR (%)	SOLEC-R (%)	SOLEC-L (%)	WALEC (%)
Normals	240[a]	5	1	5	4	7	3	4	3
Patients with vertigo	76[b]	14	18	26	22	37	23	35	29
Congenitally deaf	3	67	0	0	33	67	33	67	67
Head injury deaf	5	60	60	60	80	60	80	80	40
Unilat. labyr.-defect.	11	100	18	18	18	64	46	64	100
Menière's patients	4[c]	100	25	50	75	100	100	100	100
Bilat. labyr.-defect.	26	100	72	96	96	100	100	100	100

[a] N = 198 on SOLEC-R and SOLEC-L, N = 147 on WALEC.
[b] N = 31 on SOLEC R and SOLEC-L, N = 38 on WALEC.
[c] N 3 on SOLEC L and WALEC.

Table 5 *Group differences in relationships of ataxia test battery scores to threshold caloric respon*

NA – Not applicable. Column A – Percentage of subjects with abnormal ataxia test scores who also had abnorm threshold caloric test responses. Column B – Percentage of subjects with abnormal threshold caloric test respon who also had abnormal ataxia test scores

	Rail ataxia test battery								Floor ataxia test battery					
	Walk E/O		Stand E/O		Stand E/C		SR		SOLEC R		SOLEC L		WALE	
Subject groups	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Normals	0	0	17	17	10	8	11	17	13	8	10	8	0	[illegible]
Patients with vertigo	21	27	30	55	35	55	21	55	14	14	9	14	9	[illegible]
Congenitally deaf	NA	0	NA	0	100	50	100	100	100	50	100	100	100	[illegible]
Head injury deaf	100	100	100	100	75	100	67	67	75	100	75	100	100	[illegible]
Unilat. labyr.-defect. (acoustic-neuroma)	100	18	100	18	100	18	100	64	100	46	100	64	100	[illegible]
Streptomycin-treated Menière's patients	100	25	100	50	100	75	100	100	100	100	100	100	100	[illegible]
Bilateral labyr.-defect.	100	72	100	96	100	96	100	100	100	100	100	100	100	[illegible]

conversely the percentage of subjects in each group with abnormal threshold caloric test responses who also had abnormal ataxia test scores are indicated in Table 5

Abnormal ataxia test scores were found to be highly predictive of abnormal threshold caloric test responses not only of individuals with bilateral or unilateral labyrinthine defects but also of the congenitally deaf and head injury deaf

Moreover more than twice the percentage of patients with vertigo who had abnormal ataxia test scores than of normal individuals who had such scores were identified also as having abnormal threshold caloric test responses. In the groups of UL-Ds, Menière's patients, and BL Ds predictability was perfect, i.e. abnormal scores on each of the seven items comprising the rail and floor ataxia test batteries showed maximum (100%) prediction of abnormal threshold caloric test responses. In all five groups of subjects in which VIIIth nerve involvement was certain (patients with vertigo and the normals excluded) the WALEC test was the best single predictor of abnormally reduced sensitivity to caloric stimulation of the vestibular organ.

Similarly abnormal threshold caloric test responses were highly predictive of abnormal ataxia test scores (B columns, Table 5) In the group of Menière's patients and in the grou of BL Ds the prediction was perfect in th four items making up the floor ataxia test bat tery and was substantial (25% to 96% agree ment) on the rail ataxia test battery In th remaining three groups in which VIIIth nerv involvement was certain (UL D HID an CD) 18% to 100% of subjects with abnorma threshold caloric responses also had impaire postural equilibrium. An impressive 14% t 55% of patients with vertigo who were identi fied as having abnormal caloric thresholds als were identified as being ataxic, whereas only 0% to 17% of the normals were so identified.

Generally scores on the WALEC and SR tests were the best predictors and those of the Stand E/O and Walk E/O tests the poorest predictors of abnormal caloric responses. It is apparent also from results in Table 5 that abnormal ataxia test scores predicted abnormal caloric responses better than abnormal caloric responses predicted abnormal ataxia test scores.

It is of further interest to note that, in the group of patients with vertigo those who had a ≤5th percentile level caloric response unilaterally did not differ in the extent of their ataxia (frequency of abnormal scores on items of the test batteries) from those who had ≤5th percentile level caloric responses bilaterally

Thus from the standpoint of an ataxia test, bilateral loss was equivalent to unilateral loss of horizontal canal sensitivity to threshold caloric stimulation. Similarly in this same group, comparison of the most ataxic individuals on the basis of the percentage of ataxia tests on which scores fell < 5th percentile level revealed no systematic differences in caloric thresholds in the (1) less sensitive ear (2) more sensitive ear (3) mean responses of both ears, (4) between-ear differences in responses, and (5) frequency of unilateral versus bilateral responses < 5th percentile level. It is noteworthy also that the levels of threshold caloric responses in this group (Table 2) were independent of age and sex influences.

COMMENT

The ability of the threshold caloric test and the ataxia test battery mutually to identify those individuals with severe unilateral or bilateral labyrinthine defects was of a high order At the lowest 5% of the caloric test and ataxia test score distributions, at least, ataxia test performance skills appear to have a common relationship with sensitivity of the horizontal semicircular canals to caloric stimulation. In larger samples of individuals who have various loss of labyrinthine function, it would be useful to use other cut-off criteria, ranging from the lowest 1% to the lowest 15% or more. In large samples, moreover correlation, including multiple correlation, procedures would permit determination of the minimum number of ataxia tests and the best combination of those tests that could reliably predict, and be predicted by threshold caloric test responses From present indications, it appears that results on the tests performed with eyes closed, particularly walking, would be expected to yield the highest relationship to results of threshold caloric testing. Exploitation of such relationships in the Otoneurology Clinic shows promise of having considerable practical value.

The threshold caloric response standards set forth in Table 1 show that 95% of normal" individuals had threshold values between ≥35.4 C in the more sensitive ear The latter is identical to the average response found earlier in both ears in a different, although probably equivalent, sample of individuals (McLeod & Meek, 1962) The 0.9 C difference in thresholds between the more sensitive and the less sensitive ear at the lowest 5% of the distributions and the lesser difference in thresholds between ears at other points on the distributions (0.5–0.6 C) if shown to be reliable upon testing of larger samples of individuals, may have both practical and theoretical implications. In addition to a need for statistical crossvalidation, clinical validity of caloric findings at the lowest ends of the distribution is desirable.

Among the twelve normal individuals having the lowest responses in the less sensitive ear four were Navy deep sea divers who have descended to 200 feet using only scuba gear· one suffered a ruptured ear drum due to blocking of the Eustachian tube during a low pressure chamber descent and one had sustained a broken neck in a racing car mishap many years previously The remaining six individuals had either slight unilateral hearing loss, as established by audiometric testing, or complete freedom from any clinically detectable otoneurological defect.

The identification of far fewer patients with vertigo than individuals with pronounced labyrinthine defects as abnormal on the ataxia test or caloric test reflects the fact that such patients represented heterogenous types and severity of otoneurological disturbances or were not free of other medical problems, and, therefore, could not be grouped by any single criterion other than having been referred by an otologist or neurologist. The diagnostic classifications of those having abnormal caloric thresholds included Menière's syndrome (three), vestibular neuronitis (one) streptomycin deafness (one), severe unilateral deafness (two) paroxysmal positional vertigo (one), and vertiginous epilepsy" (one)

Although our sample of normal individuals

might be regarded as being in better health and physical condition than a random sample of individuals from a general, nonmilitary population, normality vs. abnormality of the vestibular organs however did not operate as a *selection factor Hence our sample is probably* representative of normals in terms of vestibular functions. But to an extent that "supernormality" was a significant factor in the caloric ataxia test relationships demonstrated most likely an even larger percentage (than shown) of our subjects with various losses or disturbances of vestibular functions would have been classified as abnormal on either or both types *of vestibular tests employed in this study*

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ZUSAMMENFASSUNG

Die meisten Personen, die unter so schweren Defekten des Labyrinths leiden, dass Resultate der Ataxieteste i[illegible]alb der niedrigsten 5% der normalen Verteilegen, zeigen Resultate in demselben Bereich einem kalorischen Schwellentest. In ähnlicher zeigten beinahe ebenso viele Personen mit niedrigeren Resultaten im kalorischen Schwellentest auch dürftige Resultate im Ataxietest. Obwohl die vestibularen Einflüsse, welche die Resultate der kalorischen Schwellenteste beherrschen, sehr viel spezifischer sind als die, welche die Resultate der Ataxieteste beherrschen, deutet die starke Übereinstimmung der einzelnen Resultate, in Gruppen von Personen mit verschiedenartigen Verletzten oder Störungen der Labyrinthfunktion, auf einen sehr bedeutenden gemeinschaftlichen Effekt hin.

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ULTRASONIC RECORDING OF THE VIBRATING VOCAL FOLDS

A Preliminary Report

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The ultrasonic echo method has been used to study the vibratory movements of the vocal folds. A special transducer developed for this purpose was used together with commercial ultrasonic equipment designed for heart diagnosis. Well-defined curves of the movement of the vocal folds have been recorded, which confirm the results obtained by other methods. The advantages of the ultrasonic method over other methods are: (a) continuous recording of vibrating vocal folds, (b) no interference with the articulatory movements of the tongue and the mandible, ( ) no discomfort to the patient, and simplicity of application. The method can be expected to improve appreciably in definition and resolution if specially designed ultrasonic equipment is used.

In the study of normal speech as well as vocal disorders the vibratory movements of the vocal folds are of great interest. Several methods have been developed with the object of visualizing the rapid vibratory movements of the vocal folds.

Some techniques are based upon optical methods. The synchronstroboscope belongs to this group and is often used in the clinical routine when examining patients suffering from hoarseness or other vocal disorders (Timcke, 1956 Van den Berg, 1959 Schönhärl, 1960). The method is limited, however because no continuous recording can be made of the vibratory cycles but only short fragments from consecutive cycles, which are brought together and give slow "false" motion pictures of the fast vibratory events in the larynx. Two other optical methods are the electron flash technique (Smith, 1954) and the high-speed film technique (Farnsworth, 1940; Luchsinger 1954 Moore & von Leden, 1956 and 1958). Both methods employ a laryngeal mirror and no articulatory movements can be made during the examination. In some persons, the anatomy of the pharynx and the larynx also restricts the possibilities for visualizing the vocal folds with the aid of the laryngeal mirror and these two methods are not used for routine examination of the vibrating vocal folds.

Another optical method is the photo-electric glottography (Sonesson, 1959 and 1960) in which light, being transilluminated through the skin of the neck, is allowed to pass through the glottis and is picked up by a light-conductive rod introduced into the mouth. When the vocal folds vibrate, the glottis is alternately closed and opened, and the intensity of light varies corresponding to the actual glottal area. The light-conductive rod is connected to a multiplier phototube, and on a cathode-ray oscilloscope, a curve is then obtained which corresponds to the vibrations of the vocal folds.

In 1957 Fabre introduced electroglottography. This method uses a very weak high frequency current (200 000 cps) which is allowed to pass transversely between two electrodes applied to the neck on either side of the larynx. The variation in intensity of current, varying inversely with the impedance of the tissue i.e. the vocal folds being in contact

each other or being separated, is presumed to reflect the vibratory movements of the vocal folds. An improved version of the original Fabre electrical glottograph has been found to give promising results, especially when studying certain parts of the vibratory cycle such as, for example, the transition from open to closed phase (Fant et al 1966) An acoustical method for studying the vibrations was introduced by Fant in 1961 called the inverse filtering or antifiltering technique The method is based on the principle that the formants are cancelled from the speech sound and give a signal which is presumed to be the primary larynx sound source This, in fact, reflects the volume velocity of air passing the glottis. In experiments combining the inverse filtering and the photoglottographical methods, it could be shown that the inverse filtering method gave a signal very similar to the glottographical signal (Fant & Sonesson, 1962)

It must be stressed that great care should be observed when recording the speech sound, and so far the experience of the method in routine examinations is limited.

In recent years, roentgenographic studies have been made for recording the vibratory pattern of the vocal folds (Hollien & Curtis, 60 Hollien et al 1968) Especially stroboscopic laminography proved to be useful in the ctional study of the larynx, as shown by Hollien et al. (1968) Delicate movements of the vibrating vocal folds cannot yet be recorded roentgenographically

In a short paper Mensch (1964) presented an ultrasonic recording of the vibrating vocal folds. Unfortunately the traces do not permit any analysis of the vibratory movements involved

In the present paper a preliminary report will be given of a laryngologic ultrasonographic method for studying the vibratory movements of the vocal folds. The advantage of this method is that a continuous recording can be made of the vibrations without interfering with the articulatory movements and without putting any disturbing instruments into the mouth or pharynx. In fact, the ultrasonic technique is very suitable for studying the larynx because it contains air which gives a maximum reflection of the ultrasound beam.

METHOD

The ultrasonic echo method has been used for the study of the vibrating vocal folds. The measurements are made with a modern version of the ultrasoundreflectoscope developed for nondestructive testing of materials (Firestone, 1945) This technique uses short ultrasound-pulses generated by an electrically excited ultrasoundtransducer and delivered to the material under investigation. This is done by pressing an ultrasonic transducer directly against the surface of the material under investigation. A good acoustical contact is secured by using a thin intermediate layer of oil or ultrasonic coupling jelly between the transducer and the surface of the material

Each time an acoustic pulse is transmitted, the electron beam of a cathode ray tube encased in the reflectoscope starts moving from the left to the right side of the screen in the x-direction. At the same time, the beam is deflected in the y-direction during the emission of the sound pulse If the material contains any boundaries which are impinged upon by the sound pulse and which reflect part of the sound back in a direction opposite to its original, this reflected ultrasound pulse (echo) will reach the transducer which then acts as a microphone and converts the sound pulse into an electrical pulse This pulse, after amplification, again deflects the electron beam in the y-direction. Since normally both the sound and the electron beam are moving at constant speed, the distance to the reflecting boundary can be measured in this way The method is thoroughly discussed in the paper by Hertz (1967)

Physical Properties of Ultrasound

Before the technical details and the application of the ultrasonic echo method for studying the vibrating vocal folds are described, a

short description of the physical properties of ultrasound will be presented. This might be necessary for a proper understanding of the problems involved.

When a plane acoustic wave travelling in a medium impinges upon the boundary of a second medium, part of the wave is reflected into the first medium. The reflected sound intensity I resulting from the sound beam of intensity I falling perpendicularly on a flat surface is given by

$$I = I_0 \left(\frac{\varrho_1 v_1 - \varrho_2 v_2}{\varrho_1 v_1 + \varrho_2 v_2} \right)^2 \qquad (1)$$

when ϱ_1, ϱ_2 and v_1, v_2 are the densities and sound velocities in the media 1 and 2 on both sides of the reflecting boundary. An extensive treatment of this equation can be found in most textbooks on ultrasound (Bergmann, 1954; Kinsler & Frey, 1962). Table 1 gives approximate values for ϱ and v of substances important in the special problem treated here. By comparing equation (1) with Table 1 it can be seen that very good sound reflection will be obtained from boundaries between tissue and air and to a lesser extent from boundaries between tissue and bone. From this point of view the vocal folds are excellent targets for ultrasound detection from the lateral side of the neck, as the larynx is filled with air and thus very strong ultrasound reflection from the boundary vocal fold air is to be expected.

The absorption of ultrasound is quite large in biological tissue; this factor plays an important role in ultrasonic apparatus for medical diagnosis. If a parallel ultrasonic beam passes through a certain medium, the amplitude of the particle vibration A of the beam decreases along the beam in the x-direction according to the relation

$$A = A_0 \, e^{-\alpha x} \qquad (2)$$

A_0 being the amplitude at $x = 0$, α the amplitude absorption coefficient of the medium and e the base of the natural logarithms. At the frequency 0.8 MHz a typical value of α for human muscle is 0.33 cm^{-1}, which causes the amplitude to drop to half its value after about 2 cm (Hertz, 1967). The amplitude absorption coefficient is highly dependent on sound frequency and in biologic tissue, it is roughly proportional to the frequency. Equation (2) can then be rewritten as

$$A = A_0 \, e^{-kfx} \qquad (3)$$

where k is a constant, f the sound frequency and x the distance the sound has travelled in the medium. With modern reflectoscopes constructed for medical diagnosis, it is possible to use ultrasound frequencies between 1 and 2.5 MHz for application to heart diagnosis at distances between 40 and 130 mm from the chest wall. As can be seen from equation (3) it is the product of frequency and distance travelled by the sound pulse which determines the total ultrasonic absorption. In the current application, the distance between the lateral surface of the neck and the medial contour of the vocal fold is in the order of 20 mm, which makes possible the use of a higher frequency of the ultrasound in this region. Since an ultrasound beam, like every other wave motion, diverges because of diffraction, it is important to use a frequency as high as possible for good definition. The main lobe of the ultrasonic beam is confined between the angles $\pm\theta$ which are determined by the well-known Fraunhofer diffraction formula (Hueter & Bolt, 1955)

$$\sin \theta = 0.61 \frac{\lambda}{a}$$

Table 1

Substance or tissue	Temp. (°C)	Density (kg/m^3)	Sound velocity (m/s)	Acoustic impedance (10^6 M.K.S. units)
Air	25	0.0012	331	0.0004
Water	25	0.997	1 497	1 493
Blood	—	1.0	1.560	1.560
Fat (human)	24	0.928	1 476	1.370
Muscle (human)	24	1.058	1.568	1.659
Bone (human skull)	—	1 85	3.360	6.216

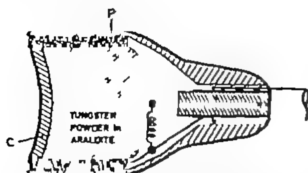


Fig 1 Transducer used in ultrasonic investigations of the vibrating vocal folds. The focused crystal, C is damped with tungsten powder in Araldite and mounted in a sound-isolating case of plastic foam, P

where λ is the wavelength of sound and *a* the radius of the circular transducer

Further for good definition of the echoes a pulse length as short as possible is desirable, since otherwise two structures lying close to each other in the direction of the beam would not be resolved, and the shortest pulse possible is just one wavelength.

Transducer

The vibratory movements of the vocal folds are rather complex, especially in low-tone frequencies. Large surfaces of the vocal folds then contact each other during the closure period of the vibratory cycle When the opening of the glottis is to occur the complete separation of the vocal folds does not take place instantly it starts from underneath, the opening progressing upwards, and not until the upper parts have separated does the glottis open (Smith, 1954) To be able to resolve movements from different parts of the vocal folds, a very narrow ultrasonic beam must be used. As has already been shown, however diffraction of the ultrasonic beam is unavoidable At 2 MHz, the wavelength of ultrasound in human tissue is about 0 75 mm. Since a narrow ultrasound beam is necessary a small transducer with a diameter of about 5 mm could be used. In this case, the ultrasonic beam diverges, as given in equation (4), with an angle of divergence of the magnitude of 10 After propagating 20 mm, i.e the distance from the lateral surface of the neck to the vocal ligament, the ultrasonic beam has spread to more than twice the diameter of the transducer A smaller crystal will give a greater angle of divergence and a larger crystal produces at the transducer surface too wide a beam. Edler (1961) discusses these problems extensively Thus, to solve this problem, ultrasound of a frequency as high as possible should be used, the frequency being limited by the sound absorption.

In order to decrease the width of the ultrasonic beam at the medial surface of the vocal fold, a transducer was used formed as a focusing bowl with a diameter of 18 mm, a focal length of 24 mm, and a resonance frequency of 2 MHz (Brush Clevite BFB/2C24-5) The material in the transducer PZT-5A, a modified lead zirconate titanate, provides a good compromise between transmitting efficiency and receiving sensitivity for the transducer together with a relatively high mechanical damping. To prevent the transducer from ringing it is necessary to mount the ceramic bowl on a backing material which will increase the damping of the disc appreciably (Fig. 1). This backing material consists of fine tungsten powder imbedded in Araldite resin, which efficiently scatters and absorbs the sound transmitted into it. The amount of tungsten powder is chosen in such a way that the acoustic impedance ρ v of the backing material is about the same as the transducer requires to reach optimum damping. A small inductance is connected in parallel with the transducer to counteract the capacitance between the silver plated sides of the ceramic bowl, used for electrical connection. With this transducer and external electrical damping, it is possible to achieve sound pulses as short as 1 or 2 wavelengths.

As the measuring distance in the present study is rather small, it should be possible to use higher ultrasound frequencies without risk of excessive absorption of the ultrasound. This would decrease the size of the transducer and

the width of the ultrasonic beam at the vocal fold. Further the pulse length could also be decreased. Because of this, the resolution of the method would increase appreciably

APPARATUS

The "Ekoline 20" ultrasoundreflectoscope, manufactured by the Smith Kline Instrument Company Philadelphia, USA, was used in this investigation. The pulse repetition frequency for this apparatus was 1000 Hz, and the pulse length was variable between 0.3–3 × 10⁻ sec. The ultrasound frequency used was 2 MHz. The apparatus was equipped with some elec tronic facilities for signal processing such as time-varying gain (depth-compensation) to compensate for ultrasound absorption in tissue and possibility for delayed sweep which makes it possible to study echoes along the x-axis enlarged and in detail. The detector circuit was of differentiating type, which decreases the rise time of the presented echoes to less than 50 nanosec, despite the used ultrasound fre quency 2 MHz, in itself suggesting a rise time of about 100 nanosec. This very fast rise time gives the reflectoscope a relative resolution of about 0 1 mm in tissue. Finally the machine provided small calibrated markers on the screen for direct calibration in tissue depth.

Because of the rapid vibratory movements of the vocal folds, only information of limited value can be obtained by direct inspection of the reflectoscope display Thus, the movements of the echoes have to be recorded in the same way as is done in the ultrasound-echo technique for heart diagnosis. For the recording of ultrasound cardiograms, three different techniques are in use the photographic method, the direct recording method, and intensity modulation of cathode ray tube (Hertz, 1967). As the vibratory frequency of the vocal folds is much higher than the frequencies oc curring in heart recording, the intensity modulation method has been used in this invest igation. Fig. 2 shows that, in this method, the intensity of the electron beam in a cathode

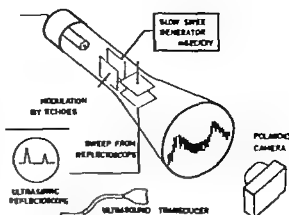


Fig 2 Block diagram of the recording method in which the ultrasonographic recordings of the vibrat ing vocal folds are directly displayed on the screen f cathode ray tube and photographed with Polar oid camera.

ray tube is modulated by the echo-signals from the reflectoscope in such a way that it appears on the screen only when an echo is present (Hertz & Edler 1956) The sweep voltage from the ultrasound reflectoscope is used to deflect the electron beam along the negative y-axis for every ultrasound pulse. Now if the time base of the cathode ray oscilloscope is running at a suitable deflection speed, say 5 to 10 millisec/div., the movement curve of the vocal fold can immediately be observed on the screen. To get better amplitude of the movement curves a delayed sweep can be used to enlarge the wanted portion of the echo on the CRT-screen. For measurement, the screen can be photographed with a Polaroid camera.

In the arrangement used, a Tektronix 566 double-beam oscilloscope has complemented the reflectoscope for the time-motion representation of the movements of the vocal fold.

The main drawback of this arrangement is the relatively low frequency of the transducer which could be higher in order to reduce the physical size of the transducer This would give a smaller ultrasound beam at the medial surface of the vocal fold. The repetition pulse frequency of 1000 Hz of the reflectoscope used gave only about ten measurement points in

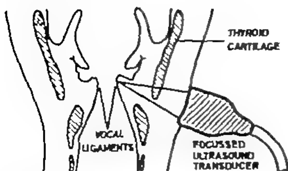


Fig 3 Schematic picture of the application of the transducer to the neck of the patient for recording movements of the vibrating vocal folds.

each vibratory cycle, which is rather low A better repetition frequency for this purpose would be between 6000 and 10 000 Hz, which could be obtained with modern electronic cir cuits.

The transducer probe was positioned at the thyroid lamina about 1 cm below and lateral to the thyroid prominence, corresponding to the horizontal level of the vocal folds. The probe was placed almost perpendicular to the surface of the neck as shown in Fig. 3 The male subject, who had a normal voice without any organic or functional disturbances in his larynx, was instructed to produce a sustained vowel, keeping the intensity and frequency of a constant. Different vowels were also pro- nced to see if any differences in the echo tern could be observed.

RESULTS

In Fig. 4 a laryngologic ultrasonogram is given, showing the structures within the vibrating larynx. The tone frequency of the voice was about 100 cps and the intensity medium. At the top of the figure, the echoes from the thyroid cartilage are seen, and below that, the oscillating echoes from one vocal fold. The vibratory amplitude of the vocal fold is measured to about 1 mm, and in the trace, the upper peak represents the open position of the vocal fold, and the lower part of the trace, the closed position. The regular shape of the curve corresponding to the vibratory cycles is found throughout the recordings.

In the echo trace from the vocal fold, the closed interval in each cycle can be seen as a horizontal level, more distinct in some cycles and less in others. The most distinct point in the trace is the top, corresponding to the maximally opened position of the vocal fold, whereas the moments of opening and closing the glottis are less distinctly marked.

The opening phase of the vibratory cycle is measured to about 3 msec, the closing phase to 4 msec and the closed interval is between 2 and 3 msec. This means that the open quotient is 0 7 and the speed quotient 0 8

For different vowels, the laryngologic ultrasonogram seemed to have the same appearance, but in the present study no systematic

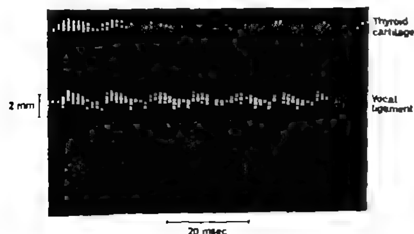


Fig 4 Ultrasonic recording of the vibrating vocal folds obtained with the pparatus shown in Fig. –

analysis was made on this problem. Further studies are needed for a more conclusive opinion.

DISCUSSION

In the present study the ultrasonic glottogram had the same appearance as that obtained with the photo-glottographic method (Fig. 4 *b*). It means that the ultrasonic method reflects the vibratory movements of the vocal folds, and especially the parts of the vocal folds having the *greatest amplitude*, i.e. the vocal ligament.

Our ultrasonic glottograms also agree well with the calculated displacement curves, which Minifie *et al.* (1967) obtained by integrating the velocity curves recorded by the Doppler frequency shift method. The present results seem to indicate that the movement of the vocal folds can be recorded by using the ultrasonic echo method, but that commercially available apparatus generates ultrasound frequencies which are too low as well as pulse rates too low to give good definition and details of the movement of the vocal folds. Because of this, it is planned to develop ultrasonic equipment especially designed for this purpose.

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ZUSAMMENFASSUNG

Die Schwingungen der menschlichen Stimmlippen wurden mittels einer ultrasonischen Echomethode studiert. Zu diesem Zweck wurde ein spezieller Transducer konstruiert, der zusammen mit einer für Herzuntersuchungen ursprünglich fabrizierten ultrasonischen Ausrüstung benutzt wurde. Wohldefinierte oszillographische Kurven der Stimmlippenschwingungen wurden erhalten. Die Vorteile der ultrasonischen Methode gegenüber früheren Methoden für das Studium der Stimmlippenschwingungen können in den folgenden Punkten zusammengefasst werden: *a*) Die Registrierung der Stimmlippenschwingungen verläuft ununterbrochen; *b*) die artikulatorischen Bewegungen der Zunge und des Unterkiefers sind unbeeinflusst, *c*) die Applikation der Methode ist einfach und bereitet dem Patienten keine Ungelegenheiten. Eine speziell konstruierte ultrasonische Apparatur für die Registrierung der Stimmlippenschwingungen kann die Methode hinsichtlich der Definition und der Resolution viel verbessern.

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THRESHOLDS FOR THE PERCEPTION OF ANGULAR ACCELERATION ABOUT THE THREE MAJOR BODY AXES[1]

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This report presents tw experiments concerned with man's sensitivity to angular acceleration about his three major body axes. The purpose of the study was to determine thresholds for accelerations bout the x, y and z axes. The thresholds of 22 normal men were established for the three axes using a precision rotation device. The angular accelerations were ordered using random, forced choice, double-stair case procedure. It was found that mean thresholds for the three axes were not significantly different and that for each of the three axes the range of individual thresholds was substantial. Intercorrelations among the three thresholds were not significantly different from zero. It was concluded that, under optimum testing conditions, the mean thresholds for rotation about the three major body axes are essentially the same, but that the threshold about any one body axis does not predict the threshold about the other two.

Most studies of the sensitivity of semicircular canals to angular acceleration have been made with the observer (O) rotating about his cephalocaudal (z or yaw) axis as reported by Clark (1967) Indeed, comparisons of the perception of angular acceleration applied about the somersaulting (pitch or y) axis and cartwheeling (roll or x) axis are so extremely limited that no definitive statements on the range of sensitivity about these axes are possible On the basis of anatomical and physiological information concerning the semicircular canals, it is quite clear that the verticals and the horizontals are functionally different in several ways as reported by Löwenstein & Sand (1940) Fluur & Mendel (1964) and Wendt (1951) For example, Löwenstein & Sand, using single nerve fiber preparations in the ray reported that the vertical canals can be directly stimulated by rotation in any plane, whereas the horizontal canals are stimulated almost exclusively by rotation about a vertical axis. They also point out that the vertical semicircular canals are phylogenetically older than the horizontal semicircular canals and are subdivided by a septum, which is not found in the horizontals. Consequently upon neurological and physiological evidence, one might expect some differences in sensitivity in rotating about the various axes. There is, however no comparative anatomical evidence for differential functioning of the three pairs of ampullar systems, furthermore, rotation about any of the three major body axes is known to stimulate more than one pair of canals.

The experimental evidence is somewhat conflicting with regard to thresholds for the perception of angular acceleration about the three major body axes. Some investigators, van Egmond *et al* (1952) and de Vries & Shierbeck (1953), have reported that there is no difference in these thresholds. Much evidence would suggest, however that rotation about the z axis will produce lower thresholds than rotations

[1] The experimental work for this study was carried out at Ames Research Center under National Aeronautics and Space Administration Grant NGL 05-046-002 to San Jose State College.

about the x or the y axes. This has been reported by Dohlman (1960) and Fluur & Mendel (1964)

Only one study was found that presents even limited evidence in the form of direct comparisons of the perception of angular acceleration in the horizontal and vertical planes on the same *Os*. Meiry (1966) studied the thresholds for the perception of angular acceleration for three normal men and reported that thresholds about the z axis varied between 0 1 and 0.2 /sec² whereas rotation of the head about the x axis produced thresholds of about 0.5 /sec² Thresholds as high as 8.2 /sec² were reported about the y axis by Sadoff *et al* (1955) but the method involved a very complex task of operating a flight simulator and no direct comparisons were made for the same *Os* for rotations about the z axis Data obtained by Decher (1963) using nystagmus as an indicator of sensitivity lent support to Meiry's data on the perception of rotation.

Decher reported that, for nystagmus, the thresholds for the vertical canals were more than twice those of the horizontal canals. On the other hand it was reported by Travis (1938) that According to our experimental re ts the two pairs of vertical canals function together are much more effective than the horizontal canals in the perception of passive rotary motion of the body

Data on differences between the functioning of the horizontal and vertical canals are also available from cupulometric studies. Some of these studies support the notion that there are no significant differences in the perception of rotation about the x y and z body axes. For example, Benson (1965) found no difference between the "cupulometric thresholds" in the x and z axes. Jones *et al* (1964) reported cupulograms that indicated similar cupulometric thresholds in pitch, roll and yaw On the other hand, they found different time constants for the duration of the after effects of rotation about the three major body axes. They reported that the duration of after effects following acceleration was greatest for yaw least for pitch, and of intermediate duration for roll. Similar results were reported by Collins & Guedry (1967), Ledoux (1958), and van der Vis (1958) Aschan & Stahle (1956) in a study of pigeons reported that there were significantly more nystagmus beats and that the duration of nystagmus was longer following the stimulation of the horizontal canals than following stimulation of the vertical canals. Collins & Guedry (1967) reported similar results for cats and humans. Fluur & Mendel (1964) studied the habituation of the horizontal and vertical semicircular canals and found that it was more difficult to produce habituation of the vertical canals than the horizontal canals as a consequence of repeated stimulation.

This brief survey of the literature makes it clear that definitive data regarding the sensitivity of man to rotation about his three major body axes are lacking. Adequate findings regarding sensitivity in these three body planes have implications for the theoretical formulation of the behavior of the semicircular canal system furthermore, they have practical implications in connection with aircraft and spacecraft flight In flying aircraft, rotations about the z axis are relatively small and infrequent, whereas rotations about the x and y axes are much more frequent and more significant for efficient flight as noted by Jones *et al* (1964) It was the purpose of the current investigation, therefore, to compare the sensitivity of normal men to angular accelerations applied around their x y and axes.

EXPERIMENT NO 1

Method

Apparatus

The Ames Man-Carrying Rotation Device (MCRD) was used to rotate the *Os*. The MCRD is a one-degree-of-freedom simulator that has been described in detail elsewhere (Clark & Stewart, 1968 *a* *b*) Accelerations may be produced and measured in 0 01 /sec² steps at the low velocities used in this study and the accelerations are programmed by an

analog computer making it possible to produce changes in acceleration with a rise time of the order of 0.1 sec. Two special seats were used to place the *O* in the proper position to rotate him about his *x*, *y*, or *z* body axis while the simulator rotated about an earth-vertical axis. In each case, the *O*'s head was positioned at the center of rotation and his legs were drawn up in a sitting position. To rotate him about his *z* axis, the *O* was placed in a normal, erect, seated position. To rotate him about his *x* axis, a chair was laid horizontally so the *O* was flat on his back with his legs in a seated position. This produced the same angular acceleration as would be found in a roll. To rotate around his *y* axis, he was rotated 90° from the previous position so that his right ear was down. Thus, with the simulator rotating about an earth-vertical axis, *O* was turned around his pitching axis. These unusual positions were necessary to maintain the direction of the *g* vector constant during rotation about the *x* and *y* axes.

Observers

Os were 18 men who were in good health by their own affirmation and by a general physical examination that revealed no significant abnormalities. They had normal hearing, and their responses to a caloric test were judged to be normal.

Procedure

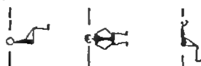
O sat with his helmet pressed back firmly in a U-shaped headrest to maintain his head in a fixed position for rotation about the *x* and *z* axes, whereas for the *y* axis, the helmet was secured in position. The angular accelerations were presented for 10 sec for all trials. The direction of the acceleration, clockwise or counterclockwise, varied at random from trial to trial, and a minimum of 30 sec elapsed between the end of one acceleration and the beginning of the next. A single series of approximately 32 trials lasted about 30 min, and at least 30 min elapsed between sessions. A 3-min rest period was given half way through each session. Preliminary practice sessions preceded the regular observations of the perception of rotation for all three body positions. *Os* were given knowledge of results in the preliminary practice trials, however, during the regular trials they had no knowledge of results. All data were collected for each *O* at a given body position before data were collected on another position. The order of the collection of data for body position was systematically ordered among *Os* by a Latin Square procedure to reduce sequential effects.

All observations were made in darkness with both eyes closed. *Os* task was to indicate the direction of rotation by pressing a switch. The angular accelerations were presented following the forced choice, random, double staircase method that was used in previous studies (Clark & Stewart, 1968 *a*, *b*). Thirty pairs of observations were made following the final level, and the mean of these accelerations was considered to be the threshold for each condition for each *O* (Clark & Stewart, 1968 *b*).

Results

The data (Table 1) for Experiment no. 1 show that the mean thresholds about the *x* and *z* axes are the same to the second decimal place. The variability about the means is essentially the same for the *x* and *z* thresholds, and this difference is not statistically significant ($P > 0.20$). The mean threshold about the *y* axis is substantially greater, however, than the thresholds about the *x* and *z* axes, but the differences do not quite meet conventional criteria for statistical significance ($P > 0.05$, $t = 1.96$ and 2.08, respectively; df = 17). This is in part due to the very great differences among the *Os* for *y* thresholds as shown in Table 1. In this regard it is notable that *y* thresholds for individual *Os* showed the highest (2.24°/sec²) and the lowest (0.06°/sec²) threshold. The standard deviation was significantly greater about the *y* axis than about the *x* and *z* axes ($P < 0.01$ in each case). Pearson correlations were also computed between the thresholds for the *x*, *y*, and *z* thresholds (Table 1). None of

Table 1 *Thresholds for the perception of rotation about the x, y and z body axes for 18 normal men*
The thresholds are in deg/sec²



Axis of rotation

[illegible]	Threshold order	(roll)	(pitch)	(yaw)
1	[illegible]	0.19	0.06	0.27
	[illegible]	0.43	1.04	0.73
3	[illegible]	0.36	0.18	0.49
4	[illegible]	0.40	0.82	0.27
5	[illegible]	0.17	0.64	0.87
6	[illegible]	0.20	0.12	0.17
7	[illegible]	0.33	0.61	0.60
8	[illegible]	0.51	0.22	0.33
9	[illegible]	0.45	0.58	0.39
10	[illegible]	0.32	1.03	0.43
11	[illegible]	0.55	2.24	0.47
12	[illegible]	0.43	0.59	0.34
13	[illegible]	1.02	0.38	0.30
14	[illegible]	0.37	0.56	0.61
15	[illegible]	0.78	0.68	0.41
16	[illegible]	0.26	0.42	0.21
17	[illegible]	0.76	0.31	0.17
18	[illegible]	0.25	1.50	0.32
Mean threshold		0.41	0.67	0.41
Median threshold		0.37	0.59	0.38
Standard deviation		0.21	0.52	0.19
Range		0.17–1.02	0.06–2.24	0.17–0.87
Thresholds compared		x–y	y–z	y–
Pearson correlation		+0.11	−0.06	+0.26

these correlations was found to be significant ($P>0.20$ at $\Lambda=18$ for each correlation). Illustrations of the marked deviation in thresholds among the three axes are to be found in several *Os*, (e.g., 10, 11 13 18).

The Latin Square design also made it possible to examine the data to determine whether practice on threshold determination about any two axes would influence the threshold of the third. Data on threshold effects for six *Os* were available for each of the three bodily positions taken first, second, or third. If experience were an important factor in threshold determination the threshold would be expected to be lower if a particular threshold were taken second or third in order An analysis of these effects showed that the order effects were very small and not statistically significant for the *x* and *z* axes ($P>0.20$ in each case). This confirms the lack of practice effects for the axis previously reported by Clark & Stewart (1968 *b*) The thresholds about the *y* axis showed a consistent decrease from the first to the third position, but these differences also were not statistically significant ($P>0.10$) for the small number of *Os* Furthermore, if the thresholds for the *x* and *z* axis were both taken before the *y* threshold, the mean *y* threshold was very near the mean of the *x* and *z* thresholds.

EXPERIMENT NO 2

Some trial observations in connection with the first experiment suggested that there was a systematic variation in thresholds as a function of certain variations in the position of the head and body It was the purpose of the second experiment to obtain preliminary data on thresholds with accelerations about the *x* and *y* body axes with the head in five different positions and with either active or passive support of the body

Method

Apparatus

The Ames Man-Carrying Rotation Device was again employed.

Observers

The *Os* were the authors and two research assistants. All four met the same selection criteria as the *Os* in the previous experiment.

Procedure

The thresholds were determined using the staircase method with the conditions in the following sequence:

Series A Thresholds were established for the *x* *y* and *z* axes as in Experiment no 1 except that the *z* axis was always tested last.

Series B *Os* were seated in the vertical chair Rotation about the *x* axis of the head was accomplished by having *O* lean forward to place his head on a headrest so that the nose was down cf. Meiry (1966). Rotation of the head about the *y* axis was achieved in the same fashion but with the right ear down. The data for the *z* axis with the *O* seated erect were obtained from a series of thresholds determined before Series A began.

Series C The conditions in Series A were repeated for the *x* and *y* axes.

Series D *O* was strapped in the horizontal seat, which was rotated so that the head was in the nose-down position. Consequently *O* was supported by the straps rather than by the seat as in Series A.

Results

While the results (Table 2) from such a limited number of *Os* must be considered with caution, certain trends are worth noting. The most obvious findings are that the thresholds about the *z* axis are low and show little variation from one series to the other the means being identical The positions nose-up and nose-down for these four *Os* also produced consistent results, but the average threshold obtained with nose up was twice that with nose down. It is noteworthy that, contrary to the findings of the larger group in Experiment no 1 the *x* axis thresholds obtained with the *Os* on their backs were higher than their *z*-axis thresholds. The

Table 2. *Thresholds for the perception of angular acceleration about the x, y and z body axes for four normal men*
The thresholds are in deg/sec²

	X axis				Y axis			Z axis	
[illegible] Series	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*A*	*B*
H	0.18	0.11	0.31	0.09	0.[illegible]1	0.19	0.22	0.11	0.16
I	0.80	0.35	1.01	0.13	0.93	0.30	0.99	0.31	0.25
J	0.28	[illegible]	[illegible]	0.29	0.46	[illegible]	0.31	[illegible]	[illegible]
K	0.42	[illegible]	[illegible]	[illegible]	0.70	0.55	0.66	0.20	0.25
Mean	[illegible]	[illegible]	0.41	[illegible]	0.50	0.34	0.45	0.22	0.22

thresholds with the head rotating about its *y* axis also showed a tendency to be lower while *O* leaned forward and, as in the larger group, the thresholds showed substantial individual differences.

DISCUSSION

These investigations concerned the sensitivity of normal men to angular accelerations applied about their three major body axes. On the basis of data obtained it became obvious that each threshold involved the stimulation of all three pairs of semicircular canals. The mean thresholds, and their standard deviations about the *x* and axes, turned out to be essentially equal. The average threshold data were in general agreement with earlier reports by Benson (1965) and van Egmond *et al* (1952) and at variance with reports by Meiry (1966) and Travis (1938). Most of the earlier studies reported data on fewer *Os* than the present experiment; as a result, there are little data on individual differences. The individual differences among our 18 *Os* were substantial about both the *x* and axes.

The thresholds about the *y* axis deserve special comment. Although the mean *y* axis threshold was greater than those about the other two axes, the differences failed to achieve conventional levels of statistical significance. Consequently it can be said that there were no statistically significant differences among the thresholds about the three major body axes. This is supported by two other findings. First, it was found that if *O*'s thresholds about the *x* and axes were determined first, the *y*-axis threshold was somewhat lower than when it was taken first. Second, with regard to the larger variability about the *y* axis, this may be merely a function of the small number of *Os* since the Clark & Stewart (1969) study of the axis thresholds for 53 normal men reported individual differences approximately the same as those about the *y* axis. It may be that practice is of greater importance in determining thresholds about the *y* axis.

The very low correlations between the

thresholds about the x, y and axes are also noteworthy. These correlations show that no accurate prediction can be made from the thresholds determined with O seated in an erect position with respect to O's sensitivity about other body axes. Consequently, measurements about the axis reveal little regarding a particular pilot's sensitivity to the very complex angular accelerations produced in operating an aircraft or spacecraft. No doubt even less prediction is possible for the complex Coriolis accelerations produced in moving about in a rotating room or on a rotating space platform as discussed by Clark & Graybiel (1961) and Newsom & Brady (1966).

A final point regarding y axis thresholds may be made. Money & Scott (1962) in a study of postrotary nystagmus in cats found that the duration of down-beating nystagmus is longer than up-beating nystagmus. This suggests that factors other than cupular deflection determine the duration of postrotary nystagmus. Although our experiment was not designed to test the hypothesis that there is a difference between thresholds for rotation "down" and "up", a determination was made of the proportion of correct responses of the 18 *Os* for these two conditions. Analysis showed that for rotation "down" the proportion correct was 0.76, while for rotation "up" the proportion correct was 0.67. This difference was not significant, which indicates that there was no directional preponderance in these thresholds. These results did not support the notion suggested by Money & Scott (196), that there is some central nervous system mechanism producing a greater sensitivity to rotation "down". Similar analysis of x and axes data produced even smaller differences: rotation to the right about the axis, 0.71; to the left, 0.68. The results for rolling right were 0.68, and left 0.72. Thus, no obvious directional preponderance was present for any axis.

The thresholds determined with the head and body in various positions (Table 2) suggested that certain head and body positions may make some difference. The two thresholds about the axis indicated a high reliability of measurement as reported by Clark & Stewart, (1968 *b*). A high reliability of measurement was also demonstrated for the two head positions for the x axis (cf. *A–C* and *B–D* in Table 2). High reliability was also shown in the replication of the same condition about the y axis. Although the mean values supported the notion of difference due to head position, a major portion of the difference was the result of changes in one O (1); consequently these y axis mean value differences must be considered with caution.

The data, taken as a whole, demonstrate convincingly that the semicircular canal system is extremely sensitive to angular acceleration and that individual differences are marked. The data also indicate that the average sensitivity about the three major body axes is the same, and that sensitivity about any one axis cannot be predicted from any other for an individual O. This is particularly noteworthy since the x and y thresholds result from stimulation of the four vertical canals. It is clear that this sensory system is influenced by complex functions and interactions and cannot be completely understood in terms of simple cupular deflection as stated by Gibson (1966) and Groen (1965).

ZUSAMMENFASSUNG

Dieser Artikel berichtet von zwei Experimenten mit der menschlichen Empfindlichkeit der Winkelbeschleunigung um die drei Hauptachsen des Körpers. Der Zweck der Versuche war die Drehreizschwellen für Beschleunigung um die y und Achsen zu bestimmen. Die Schwellenwerte von 2_ Normalpersonen wurden mit einem Präzisionsdrehungsapparat für die drei Achsen festgestellt. Die Winkelbeschleunigungen wurden durch ein wahlloses, wahl gezwungenes, doppelstufiges Verfahren festgelegt. Es erwies sich, dass sich die mittleren Schwellenwerte für die drei Achsen nicht bedeutsam unterschieden und dass die Reichweite einzelner Schwellenwerte für jede der drei Achsen beträchtlich war. Die Verhältnisse zwischen den drei Schwellenwerten unterschieden sich nicht wesentlich von Null. Die Schlussfolgerung war, dass die mittleren Schwellenwerte für Drehungen um die drei Hauptachsen des Körpers unter bestmöglichen Versuchsbedingungen wesentlich die gleichen

sind, aber dass sich anhand eines Schwellenwertes für Drehungen um eine der Achsen die Schwellenwerte der beiden anderen nicht voraussagen lassen.

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CRESCENDO TIME FOR PER ROTATORILY ELICITED NYSTAGMUS

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Forty-four clinically healthy subjects were accelerated at 1 2, 4 and 8°/sec². Both clockwise and counter clockwise acceleration was used alternately. In each acceleration how the nystagmus increased to maximal intensity was studied. The stimulation time required for this was called the crescendo time. At 1 /sec² acceleration this crescendo time was fully 15 sec and, subsequently for increasing strengths of stimulation it became shorter and shorter. The mean values were about 7.5 sec at 8°/sec².

There were wide individual variations. At 1 /sec² the times measured were between 0–29 sec, and the higher value became throughout increasingly lower when the strength of acceleration was increased, whereas the lower value remained unchanged.

On calculating the differences between the crescendo times for clockwise and counterclockwise accelerations, values were obtained whose mean was the same for different acceleration strengths, but varied within a wider time range for the weaker accelerations.

Since repeated tests were made for each strength of acceleration it was possible to assess reproducibility. This was the same for different acceleration strengths, but a tendency to increasingly shorter crescendo times was observed at 1 /sec² when the tests were repeated. For iterated identical accelerations the crescendo time could be longest for either clockwise acceleration or counterclockwise acceleration, and only in a few cases was the condition throughout the same. Statistical analyses of the borderline values were made in such a way that variations in normal persons could be assessed.

Each deviation in the cupula results in a change in the flow of impulses found in the vestibular nerve. If the flow of impulses is changed so that it is different on both sides this leads to vestibular imbalance, which in turn influences the nuclei of the eye muscles and causes ocular deviation. This ocular deviation is interrupted by rapid centrally elicited eye movements in an opposite direction and resulting in nystagmus. Through different accelerations different deviations of the cupula can be obtained experimentally and nystagmus reactions of varying intensity elicited. A certain time has been found to elapse between the beginning of the stimulation and the onset of nystagmus. This interval is called latency time (Fluur & Mendel, 1966). After the end of the latency time there follows another time period during which the intensity of the nystagmus increases successively until its full strength, after which it either remains constant or decreases. The time from the beginning of the stimulation to full intensity of the nystagmus reflex we have called the crescendo time.

In other investigations of the course of nystagmus, how frequency increases to a maximum (culmination) and then again decreases has been studied. But the exact relation of culmination to the beginning of stimulation has not been studied (Torok, 1961).

We have studied the effect on crescendo time when different intensities of constant accelerations are employed.

MATERIAL

Forty-four persons, of both sexes, aged from 20 to 45 years were investigated. Two-thirds of them were patients from the Ear Nose and Throat Clinic, whereas the rest were medical students and nursing staff. None of them had such diseases as could be conceived of influencing the results. All had normal hearing and none of them had taken any medication for some days prior to the investigation.

Apparatus for stimulation and method of investigation

We used a rotation chair for the investigation, and the experiments were in all respects similar to those previously described (Fluur & Mendel, 1969 a b)

Evaluation and calculation

In assessing the curves what is mainly studied is, how the slow phase changes. We drew a line whose course corresponded with that of the slow phase. By extending this line its angle with the horizontal zero line could be estimated. Thus this angle was a measure of the velocity of the slow phase. After stimulation was begun a gradual increase in velocity was observed, and when this no longer increased, the crescendo time was considered to have ended. We measured the time from the beginning of the acceleration to the nystagmus beat whose slow phase had the greatest angle to the horizontal. Measurement was made within 1 sec The recordings whose crescendo time could not be satisfactorily demarcated were entirely excluded, and are shown in the tables with a − sign and if similar difficulties occurred repeatedly when assessing a subject s curves he was excluded completely

We have measured the individual crescendo for each strength of acceleration (1 2, 4 8 /sec²) with three clockwise and three ...ckwise accelerations. The mean values, standard deviations, and coefficients of variation (X' S' and C') are also included in the tables. The differences between the crescendo times of the clockwise and the counter clockwise accelerations are shown at the foot of each table A + sign means that the crescendo time was longest for the clockwise acceleration, and a − sign indicates the contrary relation.

We have also calculated the mean values (X') for the crescendo times of all the subjects at the first, second and third measurements, and the standard deviations (S') and the coefficients of variation (C') have also been shown for these values, on the right in the tables.

Finally we have calculated the borderline values which, with 95 % confidence, cover 95 % of normal persons Here we have used the formula $X \pm K \times S$ where the constant K is a statistically calculated table value and S the standard deviation, in this special case S' and X is a symbol for the arithmetic mean value. The borderline values are given to the far right in each table.

RESULTS

From the tables and the figure it is evident that the crescendo times are shorter when the strength of the stimulus increased. The mean crescendo times for 1 /sec² are 15.9 sec for clockwise acceleration and 15 1 sec for counter clockwise acceleration, but for 2 /sec² the values were slightly lower 13.5 and 12.0 sec respectively 4 /sec² gave 10.5 and 9.5 sec in group 1 and somewhat higher values, 11 4 and 10 0 sec in group 2. These values are valid for the first test in group 2. At 8 /sec² further decrease in the crescendo times occurred, and the mean value was 7 9 sec for clockwise acceleration and 7.5 sec for counterclockwise acceleration.

The crescendo times for the first test are given in the top row in each table. For clockwise acceleration at 1 /sec² the values were between 0–29 sec. For the other stimulus strengths there was also extensive scatter with values between 3 and 25 sec at 2 /sec² 3–18 sec and 3–26 sec at 4 /sec² and 3–14 at 8 /sec² Counterclockwise acceleration gave the following values. 5–29 sec at 1 /sec² 3 34 sec at 2 /sec² and 2–18 sec and 1–16 sec at 4 /sec² and 3–14 sec at 8 /sec²

The crescendo times for clockwise and counterclockwise acceleration were compared by calculating the differences between them. At 1 /sec² the mean difference for clockwise acceleration was 0.89 sec shorter than for counterclockwise acceleration. The mean differences of the other accelerations were +1 41 +0.88, +2.74 and +0.32 sec. This shows that the crescendo times for clockwise and counterclock

wise acceleration are of about the same order of magnitude, and the same correlation applies also to all accelerations used. The positive sign which is found throughout may indicate a slight tendency for the crescendo time to be shorter for counterclockwise acceleration. On examining the tables for clockwise and counter clockwise differences in detail it will be found that at 1 /sec² these vary between −29 and +14 sec. The differences at 2 /sec² lie within the area −14 to +16 sec, and at 4 /sec² −13 to +7 and −10 to +10 sec. At 8 /sec² the values were −6 to +9 sec.

Variations in repeated tests are given in each table through the individual standard deviations and the coefficients of variation. The latter varied between 0.09 and 0.88 at 1 /sec² clockwise acceleration. The values at 2 /sec² were between 0.11 and 0.87. At 4 /sec² the values ranged from 0.06 to 0.87 and at 8 / sec 0.17 to 0.76. Thus these coefficients of variation are of the same order of magnitude for all the stimuli used. The results for counter clockwise acceleration agree on the whole with those for clockwise acceleration.

From the individual results it is clearly evident that the crescendo times varied substantially on repeated stimulations. This is also shown by the arithmetic mean values (X) on the right in the tables. These values were for the crescendo times at 1 /sec² clockwise acceleration in the first test 17.2 sec, and in the 2nd and 3rd tests 16.9 and 13.5 sec. Here, on repeated stimulation, the crescendo time tends to become successively shorter but at 2 /sec² clockwise acceleration there was no similar tendency nor did this occur in connection with the other stimuli. For counterclockwise acceleration the conditions were similar with a slight decrease in the X values in the 2nd and 3rd tests, at 1 /sec². For the other stimuli the X values were throughout about the same for all three tests.

If we regard the S and C values in the same way we find slight variations in the first, second and third tests.

We have previously reported on the differ-

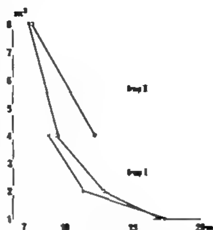


Fig. 1. Relation between strength of stimulus and duration of crescendo time. ○—○ clockwise acceleration. — counterclockwise acceleration.

ences between the crescendo times for the first clockwise and counterclockwise acceleration. The tables show that this difference was calculated for each strength of acceleration and for all three tests. For the first test at 1 /sec² the mean difference was −0.89 sec and for the following tests +2.65 and −0.25 sec. The corresponding mean differences at 2 /sec² were +1.41, +2.11 and +1.18 sec, and at 4 /sec² +0.88, +0.72 and +1.50 sec (Group 1) and in group 2, +2.74, +1.55 and −0.26 sec. At 8 /sec² the values were +0.32, ±0 and +0.68 sec. Thus it is evident that these mean values varied without any tendency to successive increase or decrease when the tests were repeated. The tables of the individual results show however that each individual can vary in his clockwise–counterclockwise difference to a considerable degree when the tests are repeated and that a large number of persons have for one test a longer crescendo time for acceleration in one direction, whereas, subsequently in the next test the crescendo time is longer for acceleration in the opposite direction. At 1 sec² for example, only 3 subjects had throughout the same clockwise–counterclockwise relations for all 3 tests. At 2 sec² the corresponding number was 8, and at 4 /sec² 5 in group 1 and 2 in group 2. At 8 /sec² only one subject had throughout a longer

Table I Group I

No. of accel.	Case no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Clockwise acceleration 1°/sec²																			
1	15	25	7	7	8	0	9	[illegible]	15	—	[illegible]	27	24	29	18	19	28	28	1
2.	17	11	6	15	17	7	9	20	17	4	19	23	28	21	15	34	21	—	[illegible]
3.	14	16	4	9	12	6	14	12	11	6	8	16	29	9	18	11	[illegible]	—	[illegible]
X	15.3	17.3	5.7	10.3	12.3	4.3	10.7	15.3	14.3	5.0	14.0	22.0	27.0	19.7	17.0	21.3	24.0	28.0	[illegible]
S	1.58	7.11	1.58	4.18	4.53	3.81	2.92	4.18	3.08	1.41	5.57	5.57	2.65	10.08	1.73	11.68	3.61	—	[illegible]
C	0.1	0.41	0.28	0.41	0.37	0.88	0.27	0.27	0.22	0.28	0.40	0.25	0.10	0.51	0.10	0.55	0.15	—	[illegible]
														X = 16.1		S = 4.30		C = [illegible]	
Counterclockwise acceleration 1°/sec²																			
1	10	19	10	10	15	29	11	8	14	5	9	29	10	28	24	29	26	26	[illegible]
2.	17	14	4	10	10	6	9	15	14	11	2	26	15	36	—	15	25	—	[illegible]
3.	1	8	5	10	9	13	10	16	21	15	13	27	20	17	—	—	20	—	[illegible]
X	9.3	13.7	6.3	10.0	11.3	16.0	10.0	13.0	16.3	10.3	8.0	27.3	15.0	27.0	24.0	22.0	23.7	26.0	[illegible]
S	8.03	5.52	3.24	0	3.24	11.79	1.0	4.36	4.06	5.05	5.57	1.58	5.00	9.54	—	9.90	3.24	—	[illegible]
C	0.89	0.40	0.51	0	0.29	0.74	0.10	0.34	0.25	0.49	0.70	0.06	0.33	0.35	—	0.45	0.14	—	[illegible]
														X = 16.1		S = 4.95		C = [illegible]	
Difference between crescendo time in clockwise and counterclockwise acceleration																			
1	+5	+6	−3	−3	−7	−29	−2	+6	+1	—	+6	−2	+14	+1	−6	−10	+2	+2	[illegible]
2.	±0	−3	+2	+5	+7	+1	±0	+5	+3	−7	+17	−3	+13	−15	—	+19	−4	—	+3
3.	+13	+8	−1	−1	+3	−7	+4	−4	−10	−9	−5	−11	+9	−8	—	—	+3	—	[illegible]
Clockwise acceleration 2°/sec²																			
1	9	25	9	18	19	9	13	—	6	3	6	11	8	13	21	20	16	12	
2.	9	20	17	8	5	11	11	7	17	3	13	26	15	18	26	11	13	[illegible]	
3.	4	20	14	11	5	13	19	11	11	12	13	25	17	16	[illegible]	11	6	15	
X	7.3	21.4	13.3	12.3	9.7	11.0	14.3	9.0	11.3	6.0	10.7	20.7	13.3	15.7	23.7	14.0	11.7	16.7	
S	2.92	2.92	4.06	5.15	8.09	2.00	4.18	2.83	5.52	5.20	4.06	8.40	4.74	2.55	2.55	5.20	5.15	5.70	
C	0.40	0.14	0.31	0.42	0.84	0.18	0.29	0.31	0.49	0.87	0.38	0.41	0.36	0.16	0.11	0.37	0.44	0.34	
														X = 13.5		S = 4.51		C	
[Count]erclockwise acceleration 2°/sec²																			
1	14	12	9	7	3	6	6	10	3	7	8	10	11	14	18	34	[illegible]	16	
2.	15	11	6	6	2	10	9	[illegible]	6	9	8	33	7	18	17	8	[illegible]	14	
3	6	4	20	20	8	9	7	14	10	15	9	12	15	9	23	—	14	21	
X	11.7	9.0	11.7	11.0	4.3	[illegible]	7.3	14.0	6.3	10.3	7.7	18.3	11.0	13.7	19.3	21.0	16.7	17.0	
S	4.95	4.36	7.38	7.81	3.24	2.12	1.58	4.00	3.54	4.18	1.58	12.75	4.00	4.53	3.24	18.38	2.35	3.61	
C	0.42	0.48	0.63	0.71	0.75	0.26	0.22	0.29	0.56	0.41	0.21	0.70	0.36	0.33	0.17	0.88	0.14	0.21	
														X = 12.1		S 5.20		C = [illegible]	
Difference between crescendo time in clockwise and counterclockwise acceleration																			
1	−5	+13	±0	+11	+16	+3	+7	—	+3	−4	±0	+1	−3	−1	+3	−14	2	−4	
2.	−6	+9	+11	+2	+3	+1	+2	−11	+11	−6	+5	−7	+8	±0	+9	+3	−5	+9	
3	−2	+16	−6	−9	−3	+4	+12	−3	+1	−3	+4	+13	+2	+7	+1	—	−8	6	
Clockwise acceleration 4°/sec²																			
1	9	8	3	7	7	6	14	5	7	[illegible]	12	15	12	11	9	5	11	11	
2.	11	4	12	9	7	11	13	8	7	16	6	16	6	6	9	12	17	18	
3.	[illegible]	[illegible]	5	15	2	[illegible]	11	—	6	14	11	23	17	7	18	4	13	12	
X	10.0	8.7	6.7	10.3	5.3	11.7	12.7	6.5	6.7	16.0	9.7	18.0	11.7	8.0	12.0	7.0	13.7	13.7	
S	1.00	5.05	4.74	4.18	2.92	6.04	1.58	2.12	0.59	2.00	3.24	4.36	5.52	2.65	5.20	4.36	3.08	3.81	
C	0.10	0.58	0.71	0.41	0.55	0.52	0.13	0.33	0.09	0.13	0.34	0.24	0.47	0.33	0.43	0.62	0.23	0.21	
														X = 10.5		S = 3.47		C = [illegible]	

X	S	C	K S	X−K S	X+K S
17.2	8.7	0.51	24.5	−7.3	+41 7
16.9	7 7	0.45	21 7	−4.8	+38.6
13 5	6 8	0.50	19..	−5 7	+32.7
15 9	7 7	0.49			
17 4	8.5	0 49	23.7	−6.3	+41 1
14 4	8.4	0.59	24.0	−9.6	+38.4
13 6	6.6	0.48	19.2	−5.6	+32.8
15 1	7.8	0.52			
−0.09	9.03		25.5	−26.4	+24.6
+2.65	8.46		24.2	−21.5	+26.9
−0.25	7.95		23.1	−23 4	+22.8
12.8	6.1	0.48	17 4	−4.6	+30.2
14 1	6.8	0.48	19.2	−5.3	+33.3
13.7	5.9	0.43	16.6	−2.9	+30.3
13.5	6 3	0.46			
11.3	7.3	0.65	20.6	−9.3	+31.9
11 9	7.2	0 60	20.3	−8 4	+32.2
12.7	5.7	0.45	16.3	−3.6	+29.0
12 0	6.7	0 57			
+1.42	7.31		20.9	−19.5	+22.3
+2.11	6.81		19.2	−17.0	+21 4
+1.18	7 42		21.2	−19 4	+23.0
9 4	3 9	0.41	11 0	−1.6	+20.4
10 4	4.3	0.41	12.1	−1 7	+22.5
11.8	5.7	0.48	16.3	−4.6	+28 1
10.5	4 6	0.43			

crescendo time for acceleration in one direction compared with that for the opposite direction.

The calculated borderline values for the crescendo times are given in each table at the far right. At 1 /sec² the upper borderline is decreased from 41 7 to 32.7 in the first and third test respectively for clockwise acceleration. Approximately the same values were found for counterclockwise acceleration. At 2 sec² the upper borderline value was 33.3 and 32.2 sec for clockwise and counterclockwise acceleration respectively The values were approximately the same at 4 /sec² in group 1 and 2 i.e. 28 1 and 25 6 sec. Finally the results at 8 /sec² show that the crescendo time according to these borderline values is less than 18 2 and 15 8 sec for clockwise and counterclockwise acceleration respectively Neither at 4 nor at 8 /sec² did these borderline values decrease when the tests were repeated.

Finally it is shown at the foot of each table on the right, that the calculated upper borderline values for the clockwise–counterclockwise difference is about 25 sec at 1 /sec² then somewhat shorter i.e. 22 sec at 2 /sec² and lastly about 15 and 11 sec at 4 and 8 /sec²

DISCUSSION

An investigation of crescendo time may appear easy to carry out. The intensity of nystagmus was found to often increase in a regular way but that the successive increases were extremely irregular in many cases. After the entire material had been examined, the characteristics were determined for demarcating the end of the crescendo time. We are aware that a large element of uncertainty had to be accepted, but since all the nystagmograms were evaluated uniformly the margin of error should probably be the same throughout.

We found that stronger stimuli cause shorter crescendo times. This is entirely in keeping with what could be expected. Rather surprising is the marked displacement towards longer times which occurs when the results at 4

No. of accel. (Case no.)	1	–	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	[illegible]

Counterclockwise acceleration 4°/sec²

1	8	5	7	2	6	6	8	[illegible]	6	12	5	9	6	12	16	—	11	13	
2.	8	7	9	16	5	9	12	7	8	13	7	8	5	13	15	7	9	17	
3	8	8	8	17	5	7	11	14	7	13	9	13	—	8	[illegible]	6	5	11	
X'	8.0	6.7	8.0	11.7	5.3	7.3	10.3	13.0	7.0	12.7	7.0	10.0	5.5	11.0	18.0	6.5	8.3	13.7	
S'	0	1.58	1.00	4.53	0.71	1.58	[illegible]	5.57	1.00	0.71	2.00	2.65	0.71	2.65	4.36	0.71	3.08	3.08	
C'	0.00	0.24	0.13	0.39	0.13	0.22	0.21	0.43	0.14	0.06	0.29	0.27	0.13	0.24	0.24	0.11	0.37	0.23	

X' = 9.4 S' = 2.11 C' [illegible]

Difference between crescendo time in clockwise and counterclockwise acceleration

1	+1	+3	−4	+5	+1	±0	+6	−13	+1	+6	+7	+6	+6	−1	−7	—	±0	−	
2.	+3	−3	+3	−7	+2	+2	+1	+1	−1	+3	−1	+8	+1	−7	−6	+5	+8	+1	
3	+[illegible]	+6	−3	−2	−3	+11	±0	—	−1	+1	+2	+10	—	−1	−5	−2	+8	+1	

Table 2. Group 2

Clockwise acceleration 4°/sec²

1	9	8	8	7	8	3	12	10	12	10	15	13	26	22	16	13	15	15	[illegible]
2.	0	6	8	9	6	21	12	4	12	8	7	12	15	17	18	23	8	16	[illegible]
3.	8	4	16	5	3	11	22	7	6	12	15	12	15	10	15	6	12	10	[illegible]
X'	5.7	6.0	10.7	7.0	5.7	11.7	15.3	7.0	10.0	10.0	12.3	12.3	18.7	16.3	16.3	14.0	11.7	13.7	11.7
S'	4.95	2.00	4.64	2.00	2.55	5.61	5.79	3.00	3.46	2.00	4.64	0.71	6.36	6.04	1.58	8.54	3.54	[illegible]	[illegible]
C'	0.87	0.33	0.44	0.29	0.45	0.48	0.38	0.43	0.35	0.20	0.38	0.06	0.34	0.57	0.10	0.61	0.30	0.33	[illegible]

X' = 11.4 S' = 3.80 C' = [illegible]

Counterclockwise acceleration 4°/sec²

1	1	5	4	7	4	13	14	16	7	7	9	12	13	12	10	11	10	14	11
2.	0.5	5	13	6	15	8	10	3	10	4	14	13	12	16	17	11	9	10	[illegible]
3.	3	11	5	7	11	13	14	13	6	6	10	7	16	26	10	6	13	14	13
X'	1.5	7.0	7.3	6.7	10.0	11.3	12.7	10.7	7.7	5.7	11.0	10.7	13.7	18.0	12.3	9.3	10.7	[illegible]	11.7
S'	1.32	3.46	4.95	0.71	5.57	2.92	2.35	6.82	[illegible]	1.58	2.65	3.24	2.12	1.41	4.06	2.92	2.12	2.35	[illegible]
C'	0.88	0.50	0.68	0.11	0.56	[illegible]	0.19	0.64	0.28	0.28	0.24	0.30	0.16	0.08	0.33	0.31	0.20	0.19	[illegible]

X' = 10.0 S' = 2.93 C' = 0.34

Difference between crescendo time in clockwise and counterclockwise acceleration

1	+8	+3	−4	±0	+4	−10	−2	−6	+5	+3	+6	+1	+13	+10	6	+2	+5	+1	1
	−0.5	−1	−5	+3	−9	−13	+2	+1	+2	+4	−7	−1	+3	+1	+1	+12	−1	+6	4
	+5	−7	+11	−2	−8	−2	+8	−6	±0	+6	+5	+5	−1	−16	+5	±0	−1	−4	3

[illegible]ckwise acceleration 8°/sec²

	7	8	9	7	6	4	10	5	13	3	9	8	13	4	5	3	10	14	9
2.	4	6	9	8	7	7	14	7	5	5	6	8	6	13	5	11	8	11	
3	5	15	1	5	10	9	7	12	3	3	13	5	12	12	8	5	7	6	
X'	5.3	9.7	10.0	6.7	7.7	6.7	10.3	8.0	7.0	3.3	9.3	7.0	10.3	9.7	6.0	6.3	8.3	10.3	[illegible]
S'	1.58	4.74	1.73	1.58	2.12	2.55	3.54	3.61	5.29	1.23	3.54	1.73	3.81	4.95	1.73	4.18	1.58	4.06	[illegible]
C'	0.30	0.49	0.17	0.24	0.28	0.38	0.34	0.45	0.76	0.37	0.38	0.25	0.37	0.51	0.29	0.66	0.19	0.39	0

X' = 7.8 S' = 2.91 C' 0

Counterclockwise acceleration 8°/sec²

1	3	14	10	4	6	4	10	9	4	6	4	9	11	8	6	8	10	9	
2.	4	8	5	7	8	6	8	8	6	9	9	6	14	9	9	5	8	9	
3.	6	9	8	6	9	2	11	6	8	6	5	10	9	7	9	6	9	10	
X'	4.3	10.3	7.7	5.7	7.7	4.0	9.7	7.7	6.0	7.0	6.0	8.3	11.3	8.0	8.0	6.3	9.0	9.3	6
S'	1.58	3.24	2.55	1.58	1.58	2.00	1.58	1.58	2.00	1.73	2.65	2.12	2.55	1.00	1.73	1.58	1.00	0.71	1
C'	0.37	0.31	0.33	0.28	0.21	0.50	0.16	0.21	0.33	0.25	0.44	0.26	0.23	0.13	0.22	0.25	0.11	0.08	0

X' = 7.5 S' = 1.79 C' 0.[illegible]

Difference between crescendo time in clockwise and counterclockwise acceleration

1	+4	−6	−1	+3	±0	±0	±0	−4	+9	−3	+5	−1	+2	−4	1	−5	±0	+5	
2.	±0	—	+4	+1	−1	+1	+6	−1	−1	−4	−3	+2	−8	+4	−4	+6	±0	+2	
3.	−1	+6	+4	−1	+1	+7	−4	+6	−5	−3	+8	−5	+3	+5	−3	−1	−2	−4	

X''	S''	C''	$K\ S$	$X-K\ S$	$X+K\ S$
8.8	4.3	0.48	12.3	−3.5	−21.1
9.7	3.7	0.38	10.4	−0.7	−20.1
10.2	4.7	0.46	13.4	−3.2	+[illegible]3.6
[illegible]	4.2	0.44			
+0.88	5.34		15.3	−14.4	−16.2
0.77	4.39		12.4	−11.7	+13.1
1.50	4.82		14.0	−12.5	+15.5
12.2	5.4	0.44	15.0	−2.8	+27.[illegible]
11.3	6.0	0.53	16.7	−3.4	+28.0
10.6	4.8	0.46	13.4	−2.8	+24.0
11.4	5.4	0.48			
9.5	4.1	0.43	11.4	−1.9	+20.9
9.8	4.5	0.46	12.5	−2.7	22.3
10.8	5.3	0.49	14.8	−4.0	25.6
10.0	4.6	0.46			
2.74	5.32		14.8	−12.1	+17.5
1.55	5.39		15.0	−13.5	16.5
−0.26	6.50		18.1	−18.3	17.9
7.7	3.4	0.43	9.5	−1.8	17.2
7.7	2.8	0.36	7.8	−0.1	15.5
8.2	3.6	0.45	10.0	−1.8	18.2
7.9	3.3	0.41			
7.4	3.0	0.40	8.4	−1.0	15.8
7.7	2.2	0.29	6.1	−1.6	13.8
7.5	2.2	0.29	6.1	−1.4	13.6
7.5	2.5	0.33			
0.32	3.89		10.8	−10.5	11.1
+0	3.57		10.0	−10.0	10.0
0.68	4.26		11.8	−11.1	12.5

acceleration are compared in groups I and II. This is partly due to the material being different in the two groups and partly to a certain resistance in the reflex arc which often seems to be present in the first test. It is evident from the tables that the crescendo time can only last one or more seconds with all the stimuli used. Several of the subjects, however had a successive increase in nystagmus for a considerably longer time. On the other hand, there were cases where there was a sudden onset of fully developed nystagmus after a long latency time. In these cases it appears as if the nystagmus reflex is strongly inhibited and then this inhibition suddenly disappears resulting in a rapidly increasing, or even from the outset fully developed, nystagmus.

For each strength of stimulus we have calculated the upper borderline values for the crescendo time. There borderline values are distinctly lower when stronger stimuli are used. With the aid of these maximal values it should be possible to determine whether an individual exhibits a normal crescendo course or deviates from the normal. At 8 /sec² for example, the crescendo time should have ceased after 18 sec.

The scattering of the values for the difference between the crescendo time for clockwise and counterclockwise acceleration is so great in relation to the mean difference that we do not venture to draw any conclusions with regard to the decrease in the S'' values which occurs when the strength of the stimulation is increased. Clockwise–counterclockwise differences are more scattered for the longer crescendo times, which are obtained with weaker accelerations and it can be assumed that this is due to the great central nervous influence which is often observed in weak vestibular stimulation.

In order to evaluate the reactions of the subjects to repeated stimuli the coefficients of variation were calculated. The mean of these individual coefficients of variation was about the same for all strengths of acceleration, which we interpret as signifying that repeated investigations give a result with the same relative variation. Thus strong stimulation does not

produce constant reactions, but also here central nervous influence plays an essential part. The successive decrease of the X' values on repeated accelerations at 1 /sec^2 may be due to greater "resistance" in the reflex arc at the beginning of the investigation.

On going through the tables for clockwise–counterclockwise differences we previously described these differences as alternately positive and negative. It seems that a normal person often displays such variations on repeated stimulations, and that a constant, throughout markedly positive or negative, clockwise–counterclockwise difference would indicate a vestibular abnormality

In animal experiments the peripheral semicircular canals, when isolated from the central nervous efferent influence, give rise to the same changes in the flow of impulses, on repeated identical stimulations (Gleisner & Henriksson, 1964) and a similar mode of functioning can be expected to occur in man In those cases where identical stimulation causes varying response this is probably due to central nervous activity

ZUSAMMENFASSUNG

Man akzelerierte rotatorisch 44 gesunde Versuchs-...n mit Stärken von 1 2, 4 und 8°/sek² Es ...de eine Beschleunigung nach rechts und nach ... gewählt. Bei jeder Akzeleration studierte man, ...ie der Nystagmus bis zur höchsten Intensität zunahm. Die hierzu erforderliche Stimulationszeit benannte man Einschwingungszeit. Bei einer Beschleunigung von 1 /sek² war diese Einschwingungszeit gut 15 Sekunden, und wurde dann, bei zunehmender Stimulationsstärke, immer kürzer Die Mittelwerte lagen ungefähr bei 7.5 Sekunden bei 8°/sek

Die Variationen zwischen den verschiedenen Individuen waren gross. Bei 1 /sek² wurden Zeiten zwischen [illegible] und 29 Sekunden gemessen, und der höhere Wert wurde durchgehend immer kleiner bei zunehmender Akzelerationsstärke, während der niedrigere Wert durchgehend unverändert blieb.

Bei Berechnung der Unterschiede zwischen Einschwingungszeiten bei Rechts- und Linksbeschleunigung erhielt man Werte die im Durchschnitt gleich waren bei verschiedenen Akzelerationsstärken, aber bei den schwächeren Akzelerationsstärken innerhalb eines grösseren Zeitumfangs sehr variierten.

Da bei jeder Akzelerationsstärke mehrere Teste gemacht wurden, konnte man die Reproduzierbarkeit beurteilen. Diese ist die gleiche bei verschiedenen Akzelerationsstärken, eine immer kürzere Einschwingungszeit wurde jedoch bei 1 /sek² notiert, als man die Teste wiederholte. Bei wiederholten gleichen Akzelerationen konnte die Einschwingungszeit am längsten sein, entweder bei Rechts- oder auch bei Linksbeschleunigung, und nur in wenigen Fällen war das Verhältnis durchgehend gleich.

Statistische Berechnungen der Grenzwerte sind aufgestellt worden, sodass eine Beurteilung der Variationen bei einem Normalmaterial gemacht werden konnte.

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DYNAMIC EQUILIBRIUM IN SQUIRREL MONKEYS AFTER UNILATERAL AND BILATERAL LABYRINTHECTOMY

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The maintenance of dynamic equilibrium in the squirrel monkey measured by squirrel monkey rail test, was investigated after unilateral, bilateral simultaneous, and bilateral non-simultaneous labyrinthectomy After unilateral labyrinthectomy most cases regained the pre-operative threshold level within 40–90 days postoperatively After bilateral ablation of the peripheral labyrinth, whether simultaneous or not, the performance threshold of the squirrel monkey rail test severely declined. Therefore, it is concluded that the existence of peripheral vestibular labyrinth is essential for the maintenance of dynamic equilibrium when the condition requires advanced psychological and/or physical skill. Inasmuch as the dynamic balancing activity in daily life does not usually require advanced psycho-physical training and skill, the animals did not demonstrate any difficulty in their daily activities in the cages.

The physiological contribution of the peripheral labyrinth is most important for the maintenance of dynamic equilibrium. Although postlabyrinthectomy spontaneous (or compensatory) nystagmus has been studied by many investigators since the time of Bechterew the investigation of postlabyrinthectomy equilibrium and locomotion has not been carried out in a quantitative fashion, in experimental animals.

A method to examine dynamic equilibrium in squirrel monkeys in a quantitative fashion was previously developed by the author (Igarashi, 1968) The purpose of the present study is to investigate the mode of equilibrium compensation after unilateral or bilateral (simultaneous or non-simultaneous) destruction of the peripheral labyrinthine end organs, in the squirrel monkey

PROCEDURE

Squirrel monkeys, about 1 /₂ years old, were used in the present study Eleven squirrel monkeys were used for investigation of the effects of unilateral labyrinthectomy One of these was used later for bilateral non-simultaneous labyrinthectomy before complete recovery from the first operation had occurred. Therefore the data from ten subjects was collected after unilateral labyrinthectomy Three squirrel monkeys were subjected to bilateral simultaneous labyrinthectomies while five other squirrel monkeys had bilateral but non-simultaneous labyrinthectomies (in four of these animals the second operation was performed after complete recovery of dynamic equilibrium from the first unilateral labyrinthectomy while in the other one the second operation was performed when his postoperative threshold was still midway (700) to the level of pre-operative threshold)

All labyrinthectomies were performed with routine sterile procedure through a retro-auricular approach to the labyrinth.

The postoperative compensatory pattern of

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dynamic equilibrium was investigated according to the squirrel monkey rail test procedure which was previously described by the author (Igarashi, 1968) Postoperative threshold measurements were performed 2–3 times per week. In addition to the measurements of the post operative daily threshold, the daily condition of ataxia was studied by scoring the percentage of total daily falls/total daily trials.

The number of days required for compensation to take place was determined statistically by using *t*-test. The pre-operative mean threshold and mean percentage of falls (condition of ataxia) were determined by taking the average of each of these two measures for all subjects during five tests seasons preceding the labyrinthectomy A distribution was obtained by combining each individual subject s five session pre-operative average as a single score. Postoperatively raw data scores for all subjects were combined within 3-day blocks to form comparison distributions (one comparison distribution for every 3 days postoperatively) The duration for complete functional compensation was determined by successively comparing each 3-day comparison distribution with the pre-operative distribution until the *t* value obtained was no longer significant at the 0 05 level of probability (one tail)

Upon the attainment of complete compensa- after unilateral procedure or when the t ys failed to acquire any significant compensation for 3 to 6 months after bilateral procedure, the animals were sacrificed by means of intravital cardiac perfusion. The temporal bones were removed and processed according to the standard preparation procedure All specimens were examined *light-microscopically*

RESULTS

Unilateral labyrinthectomy

All subjects were extremely ataxic with spontaneous nystagmus, and most of them could not perform on the rail at all (zero meaning the subject could not traverse the rail



Fig 1 Average value of performance thresholds after unilateral labyrinthectomy from ten subjects. preoperative threshold range — mean value of pre operative thresholds.

even though it was not rotated) immediately after the unilateral labyrinthectomy These symptoms subsided gradually and about half of the total subjects regained almost $^{1}/_{2}$ of pre-operative threshold values within 21 days postoperatively Four out of ten monkeys regained their pre-operative thresholds on the rail test in an average of 40 days after the operation, ranging 30 to 51 days. The other six regained their pre-operative thresholds between 57–90 days postoperatively The average value of postoperative performance thresholds from ten subjects is presented in Fig. 1 All ten subjects in this category regained their pre-operative thresholds eventually

Fig. 2 exhibits the average value of the percentage of daily falls from the ten animals in-

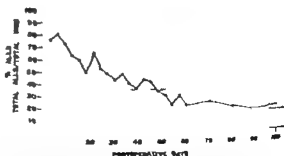


Fig 2 Average value of daily percentage of falls (which indicates the condition of ataxia) after unilateral labyrinthectomy from ten subjects upper limit of pproximate range (pre-operative); — pre-operative mean value of percentage of falls.

vestigated. The result generally agreed with that of daily thresholds.

According to the results from the *t*-test, the first comparison distribution that was not significantly different from the pre-operative distribution, was the one for 37–39 days for threshold performance, and also it was the one for 46–48 days for the degree of ataxia.

Bilateral simultaneous labyrinthectomy

The observation was made for a minimum of 120 days and maximum of 190 days after the operation. Except for one monkey which at one time had a postlabyrinthectomy threshold of 100 RPM (its pre-operative threshold was 1050 RPM) the thresholds of these monkeys remained zero, or 50 (meaning they could barely traverse the ¹/ " wide 6 long rail but they failed to do so when the rail was rotated even at the slowest speed), or 100 RPM at maximum. One monkey actually demonstrated the threshold of zero even 109 days postoperatively and the other one could not gain the threshold level of 50 RPM until his 80th postoperative day. Fig. 3 demonstrates the average value of postoperative thresholds from three animals in this group.

Bilateral non-simultaneous labyrinthectomy

The animals were studied for a period of 110 days after the second operation. Postoperatively the monkeys were extremely ataxic and showed compensatory nystagmus which gradually subsided.

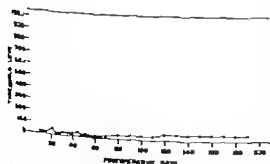


Fig. 3. Average value of performance thresholds after bilateral simultaneous labyrinthectomy (three subjects). — mean of pre-operative thresholds.

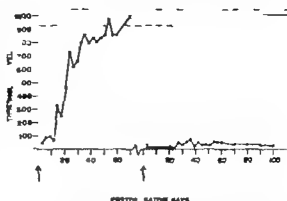


Fig. 4. Average value of performance thresholds after first (L-I) and second (L-II) operation of bilateral non-simultaneous labyrinthectomy (five subjects). → pre-operative threshold range — mean value of pre-operative performance thresholds.

The thresholds of rail performance in these animals were also extremely low immediately after the second operation, however, each one demonstrated slight recovery in its rail performance ability after the third postoperative week. One subject had thresholds of 150 RPM on one occasion about 5 weeks postoperatively, however, his threshold remained at 50 RPM during the rest of the time. All other subjects failed to gain a threshold higher than 50 RPM any of the time. One animal failed to gain the threshold level of 50 RPM until his 60th postoperative day. Average value of thresholds both after the first and second labyrinthectomies can be seen in Fig. 4.

All subjects after bilateral labyrinthectomy (whether simultaneous or not) failed to recover good performance threshold on the rail. Also, these monkeys exhibited slight ataxic gait or head shaking (or tilt), when they were forced to be in an extremely alert condition, although they did not have any definite difficulty in maintaining balance in their daily lives in the cage.

Histology

Complete destruction of the vestibular end-organs was histologically confirmed in all tem-

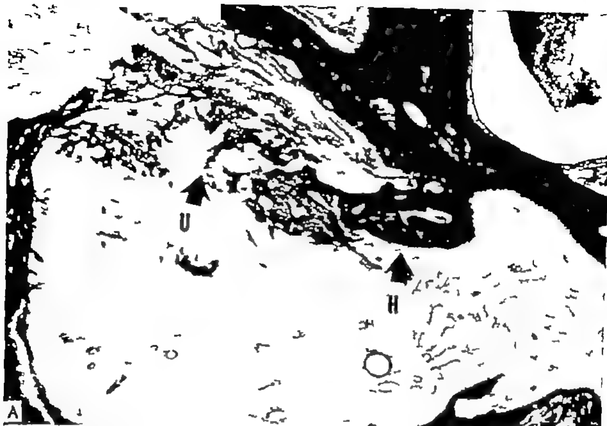


Fig 5 (A) Photomicrograph demonstrates a representative view of surgically destroyed utricular macula (*U*) and horizontal semicircular canal crista (*H*) area, 120 days after the operation. Notice extensive ossification in the area. 18.

poral bone specimens examined (Fig. 5 A, B) Vestibular ganglia (Scarpa) were found to be intact light-microscopically (Fig. 6 A, B)

DISCUSSION

The long-term results after bilateral (regardless simultaneous or not) labyrinthectomy indicated that this condition created a severe impairment in their behavioral performance for the advanced psycho-physically trained condition required in the squirrel monkey rail test, however it was found that there was no severe ataxia in their ordinary daily lives. In other words, the compensatory mechanism, which took place within the vestibular neural system (most probably vestibular nuclei and reticular formation), together with assistance from all other cues was not sufficient for the behavioral performance of this psycho-physically trained status which required good co-ordination of extremities, although it was sufficient to maintain their daily activities on the floor (in the cage) Thus, the requirement of an intact vestibular end organ for maintenance of dynamic equilibrium in the psycho-physically trained condition appear to be established by the present study Other workers have made somewhat similar observations after labyrinthectomy in untrained animals, for example, Gray & Lissmann (1947) described that a toad exhibited unco-ordinated swimming movements after bilateral labyrinthectomy Graybiel & Fregly (1966) have also found that labyrinthine defective or involved human subjects showed poor scores in quantitative ataxia test batteries.

The recovery of the rail test threshold after unilateral labyrinthectomy suggested that the presence of a contralateral vestibular labyrinth and other visual and proprioceptive cues were



(B) Photomicrograph exhibits extensive ossification in both saccular area (*S*) and in semicircular canal, 120 days after labyrinthectomy 30.

sufficient to permit complete recovery to a preoperative performance level of this advanced psycho-physical task.

Aschan *et al.* (1956) reported positive spontaneous nystagmus (when the patient's eyes were closed) many years after unilateral labyrinthectomy Goto (1964) also described similar findings and dysequilibrium (one case: post-the Romberg, 550 days postlabyrinthectomy) which was tested by goniometer. In the present study dynamic equilibrium compensation could take place faster as many other cues probably were contributing to assist the monkey to compensate, especially by frequent postoperative testing procedures, when compared to the similar postoperative status in humans. When the test was being performed, visual and proprioceptive cues fully contributed to the locomotion performance.

Precht *et al.* (1966) have termed the 30–45

A

B

Fig 6 (A) Photomicrograph demonstrates a normal view of Scarpa s ganglion and macula utriculi (*U*). 90.
(B) Photomicrograph shows a representative view of morphologically intact Scarpa s ganglion after the labyrinthectomy procedure. The area of utricle is occupied by ossification (60 days postoperative). 90.

days postlabyrinthectomy status as the "compensated stage" as induced nystagmus was diminished or almost abolished, postural abnormalities were less marked, and only a tendency to lurch to the operated side could be seen on quick body movements. The monkeys in the present study have been tested on the rail (which could also be considered to be a condition of physical training) frequently after the operation, and their recovery after unilateral labyrinthectomy could reasonably be considered to be faster because other cues were enforced, however some of them even so required considerably longer times to regain absolute pre-operative status in this performance task.

The different length of postoperative time for the absolute compensation of dynamic equilibrium was probably due to intra-individual variance, as the testing procedures were identical for all subjects. Although monkeys about 1 1/2 years old had usually been used for squirrel monkey rail test experiments, two of the unilaterally labyrinthectomized monkeys were slightly older than the others (possibly over 2 years of age) and they required a longer time to compensate. Thus, the age of the subject may be a critical factor in this sort of experiment.

In addition to recording daily thresholds, after unilateral procedure, the percentages of daily falls were studied, and the results generally agreed with each other (Figs. 1 and 2), although the results from the *t*-test showed slight difference. Thus, the performance ability on the rotating rail and the degree of ataxia (as indicated by the daily percentage of falls) are altered simultaneously.

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ZUSAMMENFASSUNG

Die Aufrechterhaltung des dynamischen Gleichgewichtes bei Saimiri sciureus (Totenkopfäffchen, Squirrel monkey), gemessen an dem Saimiri-sciureus Schienenversuch, wurde nach einseitiger, nach gleichzeitig doppelseitiger und nach nicht gleichzeitig doppelseitiger Labyrinthektomie untersucht. Nach einseitiger Labyrinthektomie kamen die meisten Fälle innerhalb von 40–90 Tagen nach dem chirurgischen Eingriff wieder auf die ursprüngliche Schwellenstufe. Nach doppelseitiger Entfernung des peripheren Labyrinths, ganz gleich ob gleichzeitig oder nicht, fiel die Ausführung der Schwelle des Saimiri-sciureus Schienenversuches scharf ab. Daraus folgt, dass das Vorhandensein des peripheren Labyrinths für die Erhaltung des dynamischen Gleichgewichtes wichtig ist, wenn die Verhältnisse erhöhte psychologische und/oder physikalische Fertigkeit verlangt. Die Tiere in den Käfigen hatten keine Schwierigkeiten in ihrem alltäglichen Tun, da die dynamische Gleichgewichtstätigkeit im täglichen Leben im allgemeinen keine fortgeschrittene psychologisch-physikalische Ausbildung und Fertigkeit beansprucht.

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D C. POTENTIALS IN THE SEMI-CIRCULAR CANAL OF THE PIGEON

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Membranous lateral semi-circular canals of pigeons were explored for measurement of D.C. potential of the endolymphatic fluid. Measurements were made relative to the perilymphatic fluid in the immediate vicinity of the exploring electrode. Explorations were made with both metal micro-electrodes and capillary micropipettes filled with 3 molar KCl. Both types of electrodes gave equivalent recordings of the D.C. potential inside the semi-circular canals. In the majority of experiments the membranous canals were opened by incision and the electrodes inserted under visual control. The D.C. potential recorded was consistently high (+25 to + 8,3 mV). In two experiments where the canal wall was penetrated by the micro-electrodes the potentials recorded were low (+1 to +2 mV). These findings are discussed.

The D C. potential in the scala media of the cochlea was first measured by von Békésy (1952) D C. potentials have also been meas ured in the vestibular labyrinth, in the utricle, e saccule and the semi-circular canals (Smith al 1958 Trincker 1957 1959 Gannon et al 1958 Schmidt & Fernández, 1962 Schmidt, 1963)

Trincker reported a potential of +30 to +40 mV in the lateral semi-circular canal of the guinea pig (Trincker 1957)

In a subsequent publication, Gannon *et al* (1958) found a much lower potential and felt that it was no more than +5 mV as recorded in the utricle. They felt that the difference in the values obtained were due to the use of dif ferent kinds of micro-electrodes. Trincker (1957) used a metal electrode presumably not as stable for D C. recordings as could be de sired, whereas, Gannon *et al* (1958) used a capillary micropipette filled with 3 molar KCl, with a 1μ tip and a resistance of 7 to 10 megohms However the discrepancies between the results of the earlier investigators did not seem to have been satisfactorily clarified (Gannon *et al* 1958) It therefore seemed to be of interest to investigate if variations in approach to the endolymph and the use of different electrodes could contribute to elucidating this problem.

METHOD AND MATERIAL

For this study we chose to use white pigeons (Columba livia domestica) weighing about 500 g. In all, 18 pigeons were used in this study They were anesthetised with an intravenous injection of Equithesin (about 1 4 cc per 1000 g) A routine tracheotomy was made. The Ewald s approach was used to expose the membranous horizontal semi-circular canal

After exposure the membranous canal was elevated out of the perilymph with a very fine wooden splinter to prevent a contact between the perilymphatic and the endolymphatic fluids. A small incision was then made in the membranous canal wall with a pair of fine iris scissors, close to the ampullated end but not in the ampulla. The electrodes were introduced into the endolymphatic fluid under direct vision with a micromanipulator For recording purposes two types of micro-electrodes were used. One was a metal electrode made of platinum iridium wire insulated with Corning solder glass, with a tip diameter of about 1μ and a

tip impedance of 600 to 700 kohms as described by Wohlbarsht *et al.* (1960) In other experiments capillary micropipettes with 1 to 2μ tip diameter filled with 3 molar KCl were used. It was connected to the voltameter by a platinum-iridium wire.

Recordings were made with a Model 203 Keitel microvoltameter The reference electrodes were placed close to the exploring electrode in the perilymphatic fluid.

In a number of these experiments the results of anoxia on the potentials were also noted.

FINDINGS AND DISCUSSION

A total of 18 experiments were carried out. There were 12 valid experiments. In the other 6 either for reasons of technical difficulty or poor condition of the animal, experiments could not be completed. Six of these explorations were carried out with a metal micro-electrode and 6 with a 3 M KCl capillary micro-electrode The first two experiments were carried out with metal electrodes which were pushed through the wall of the membranous canal These experiments yielded a value of +1 and +2 mV only The membranous canal of the pigeon is considerably thicker and tougher than those for instance of guinea pigs It is therefore very difficult to penetrate such a wall with a micro-electrode without the risk of damage to the electrode or to the canal. Dr G Dohlman therefore suggested to cut open the canal and then insert the electrode into the endolymph under direct vision. Using this technique four explorations were done with metal micro-electrodes. The average D.C. potential recorded was then +25 mV (endolymph positive with respect to perilymph) The range of variation in this group was from +20 to +30 mV (Table 1) In the 6 experiments done in the same way with a capillary micro-electrode the average value was +28.5 mV the range being +15 to +40 mV The exploring electrode was away from the area of injury potential.

The results obtained in these present experiments therefore indicate that the exploration of the D.C. potentials of the fluid within the

Table 1

Experiment no	Metal micro-electrode	Capillary micro-electrode
1	1 mV[*]	
2	+ 2 mV	
3	30 mV	
6		40 mV
7		5 mV
9		30 mV
11	30 mV	30 mV
12		30 m
14	20 mV	
17	20 mV	
18		15
Average	25 mV	28.5 mV

Endo-lymphatic potential values measured [illegible] the perilymph
[*] Excluded in the calculation of average value

vestibular labyrinth with a good metal electrode yields results as stable as with the capillary micro-electrode filled with 3 molar KCl This might suggest that the values obtained by Trincker (1957) with the metal micro-electrode in the guinea pigs may still be valid Contamination from perilymphatic fluid was if not eliminated at least greatly reduced by the use of the small wood splinter support under the membranous canal and did not seem to affect the results. The D C potentials recorded in this way could be shown to respond to anoxia With increasing anoxia the D C potential values decreased but returned to the original levels on eliminating anoxia if this was done within a few minutes. On sacrificing these animals the D C potentials went down to negative values of −10 mV relative to the perilymph.

In a comparative study of values on D.C. potentials in vertebrates Schmidt & Fernández (1962) found +5 mV D.C. potentials in the semi-circular canal of the bird. Schmidt (1963) found in the common pigeon +3 to +5 mV D.C. potential

In our experiments higher values were obtained in pigeons It is difficult to explain this discrepancy between our results and those of previous investigators. It might be important to mention that in the first two experiments where metal electrodes were pushed through the in-

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D C. POTENTIALS IN THE SEMI-CIRCULAR CANAL OF THE PIGEON

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Membranous lateral semi-circular canals of pigeons were explored for measurement of D C. potential of the endolymphatic fluid. Measurements were made relative to the perilymphatic fluid in the immediate vicinity of the exploring electrode. Explorations were made with both metal micro-electrodes and capillary micropipettes filled with 3 molar KCl. Both types of electrodes gave equivalent recordings of the D C. potential inside the semi-circular canals. In the majority of experiments the membranous canals were opened by incision and the electrodes inserted under visual control. The D C. potential recorded was consistently high (+25 to +28.3 mV). In two experiments where the canal wall was penetrated by the micro-electrodes the potentials recorded were low (+1 to +2 mV). These findings are discussed.

The D C. potential in the scala media of the cochlea was first measured by von Békésy (1952) D C potentials have also been measured in the vestibular labyrinth, in the utricle saccule and the semi-circular canals (Smith et al 1958 Trincker 1957 1959 Gannon et al., 1958 Schmidt & Fernández, 1962 Schmidt, 1963)

Trincker reported a potential of +30 to +40 mV in the lateral semi-circular canal of the guinea pig (Trincker 1957)

In a subsequent publication, Gannon *et al.*, (1958) found a much lower potential and felt that it was no more than +5 mV as recorded in the utricle. They felt that the difference in the values obtained were due to the use of different kinds of micro-electrodes. Trincker (1957) used a metal electrode presumably not as stable for D C. recordings as could be desired, whereas, Gannon *et al* (1958) used a capillary micropipette filled with 3 molar KCl, with a 1μ tip and a resistance of 7 to 10 megohms. However the discrepancies between the results of the earlier investigators did not seem to have been satisfactorily clarified (Gannon et al., 1958) It therefore seemed to be of interest to investigate if variations in approach to the endolymph and the use of different electrodes could contribute to elucidating this problem.

METHOD AND MATERIAL

For this study we chose to use white pigeons (Columba livia domestica) weighing about 500 g In all, 18 pigeons were used in this study. They were anaesthetised with an intravenous injection of Equithesin (about 1 4 cc per 1000 g) A routine tracheotomy was made. The Ewald's approach was used to expose the membranous horizontal semi-circular canal.

After exposure the membranous canal was elevated out of the perilymph with a very fine wooden splinter to prevent a contact between the perilymphatic and the endolymphatic fluids. A small incision was then made in the membranous canal wall with a pair of fine iris scissors close to the ampullated end but not in the ampulla. The electrodes were introduced into the endolymphatic fluid under direct vision with a micromanipulator For recording purposes two types of micro-electrodes were used. One was a metal electrode made of platinum iridium wire insulated with Corning solder glass, with a tip diameter of about 1μ and a

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POSSIBLE INTERACTIONS BETWEEN GRAVI RECEPTORS AND SEMICIRCULAR CANALS IN THE HABITUATION OF VERTICAL NYSTAGMUS IN PARROTS

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Horizontal ocular nystagmus from restrained birds was habituated in a directionally specific fashion by means of repeated angular accelerations. Substantial response recovery was evident following two weeks of rest. Vertical nystagmus was similarly habituated in a separate group of birds which were positioned so that vertical semicircular canals were in the plane of rotation. By changing the position of these birds 180° after the habituation trials, the same set of vertical canals could be stimulated but with the otoliths and other gravi-receptors oriented differently. Habituation was relatively specific for the direction of nystagmus repeatedly elicited and for the head and body position maintained during habituation trials. A dynamic interaction between gravi-receptors and the semicircular canals was suggested as a possible feature of nystagmic habituation.

Several studies have cited the possible influences of non-specific and specific gravi-receptors, particularly the otoliths, on reactions usually attributed solely to the activity of the semicircular canals. Thus, the failure of habituation of vestibular responses to transfer from one stimulus modality (e.g., caloric) to another (e.g., rotation) in cats (Capps & Collins, 1965 Collins, 1964 a, 1964 b Mertens & Collins, 1967), and from one quadrant of head movements to another in human coriolis studies (Guedry 1965 Guedry *et al.* 1964) has been attributed to possible differences in the pattern of stimulation contributed from several sources including the otoliths. Data obtained from rabbits which had undergone nerve resections led Owada & Okubo (1963) to conclude that the otoliths exert some regulatory functions on nystagmus, while studies with centrifuges showed that otolithic stimulation influenced the magnitude of nystagmus obtained from humans as a result of both calorizations (Bergstedt, 1961) and angular accelerations (Lansberg *et al* 1964) Linear accelerations produced by parallel swings have caused nystagmus in humans and rabbits when the eyes of the subjects were deviated laterally (Jongkees & Philipszoon, 1962, 1963 1964 Philipszoon, 1962), and horizontal nystagmic reactions have been reported for humans exposed to periodic linear accelerations (Niven *et al.*, 1965). Additionally the augmentation or reduction of nystagmus during rotation of human subjects about an earth-horizontal axis was attributed by Guedry (1966) to the changing location of the gravity vector

Collins (1969) examined the possible influence of gravi-receptors on habituation of nystagmus in cats. Cats placed on their sides were given a series of angular accelerations which significantly reduced their nystagmic reaction. However when the position of each cat was changed by 180° (thereby keeping the same sets of canals in the plane of rotation) no habituation effects were apparent during subsequent testing.

With few exceptions (e.g., Winget & Smith, 1962 Winget *et al* 1962), studies of vestibular habituation in birds have been concerned with head nystagmus rather than with ocular responses to stimulation of the semicircular canals. The present study was designed to examine the process of habituation of ocular nystagmus in birds and to explore further the possibility of an interaction between gravi-receptors and semicircular canal activity in the habituation process.

METHOD

Subjects

Habituation of horizontal nystagmus was examined in a group of six African parrots. Sixteen dwarf parrots were used to examine the influence of the position of specific and non specific gravi-receptors on the habituation of vertical nystagmus Each bird was restrained by means of a band of gauze which served both to keep the wings against the body and to secure a special arched, cloth-covered brace which extended from the back of the bird around the top of its head. The beak of the bird was taped closed and secured (in approximately the position of normal carriage relative to the bird's body) to the edge of a metal container in which the bird was placed. ...itional tape extended across the cloth-...u brace from one side of the container to ...e other and provided complete immobilization of the subject. Needle electrodes were inserted on each side of each eye of the African parrots, and above and below each eye of the dwarf parrots so that separate recordings of either horizontal or vertical components of nystagmus were obtained from the right and left eye of each bird. Recording from both eyes separately but simultaneously permitted a choice of tracings for scoring and provided additional assurance that a complete set of tracings would be obtained even though a recording problem (e g., a pulled electrode) might develop The recorder was an Offner Type R Dynograph with 3-sec time constants. Animals were tested in groups of two or three by means of a tier arrangement on the Huffman Rotation Device (Collins & Huffman, 1964 Collins & Updegraff 1966) A variety of auditory stimuli was presented during response periods to help maintain alertness in the birds.

African parrots lateral-canal stimulation

African parrots were tested with their lateral canals in the plane of rotation and with their heads (and bodies) positioned over the turning axis of the rotator Two pre and two post-habituation trials, immediately preceding or following the habituation series, were conducted as indicated in Table 1 stimuli comprised accelerations and decelerations of 5 /sec² for 12 sec separated by 2 min of constant velocity The habituation series comprised 15 accelerations of 5 /sec² for 12 sec followed by 1 min of constant velocity and subthreshold decelerations (0 15 /sec² for 400 sec) An additional pair of post-tests, conducted 2 weeks after the initial pair examined

Table 1 *Outline of test procedure for stimulation of the lateral semicircular canals in six African parrots*

Pre- and post-habituation stimuli were accelerations and decelerations of 5°/sec² for 12 sec separated by min of constant velocity Habituation stimuli comprised accelerations of 5°/sec² for 12 sec (decelerations were subthreshold)

African parrots	Pre-trials (accel and decel) 1	2	Habituation series (15 trials, accel only)	Post-trials (accel and decel) 1	2
A 1 A 2, A 3	CW	CCW	CW	CW	CCW
A–4, A 5, A–6	CCW	CW	CW	CCW	CW

Table 2. *Outline of test procedure for stimulation of the vertical semicircular canals in 16 dwarf parrots*

Pre- and post-habituation stimuli were accelerations and decelerations of 10°/sec² for 1 sec separated by min of constant velocity Habituation stimuli were accelerations of 10°/sec² for 12 sec (decelerations were subthreshold)

Dwarf parrots	Pre-1 and Post-1 (accel and decel)	Pre-2 and Post 2 (accel and decel)	Habituation series (15 trials, accel only)
D-1	CW beak down	CW – beak up	All CW (beak down)
D-2	CW – beak up	CW – beak down	
D-3	CCW – beak down	CCW – beak up	
D-4	CCW beak up	CCW – beak down	
D-5	CW – beak down	CW – beak up	All CW (beak up)
D-6	CW – beak up	CW – beak down	
D-7	CCW – beak down	CCW – beak up	
D-8	CCW – beak up	CCW – beak down	
D-9	CW – beak down	CW – beak up	All CCW (beak down)
D-10	CW – beak up	CW – beak down	
D-11	CCW – beak down	CCW – beak up	
D-12	CCW – beak up	CCW – beak down	
D-13	CW – beak down	CW – beak up	All CCW (beak up)
D-14	CW – beak up	CW – beak down	
D-15	CCW – beak down	CCW – beak up	
D-16	CCW – beak up	CCW – beak down	

the question of retention of habituation All trials were in total darkness rest intervals between trials (i.e with the rotator at a standstill) comprised 3–5 min in room illumination

Dwarf parrots, vertical-canal stimulation

In the major portion of the study dwarf parrots were placed in a tilted position with their heads over the axis of rotation. Two body placements were used, "beak down and "beak up For the "beak down" position, the container holding the bird was tilted 90 forward and approximately 45 to the left of the bird (from the restrained, upright position) the "beak up position represented a change of 180 from "beak down In these positions, a brisk vertical nystagmus could be elicited. By visual observation of birds used in pilot studies, the direction of the eye-movement responses tended to be somewhat oblique and the direction of the fast phase in one eye was opposite to that of the other eye.

The 16 dwarf parrots were divided into four groups according to body placement and direction of rotation during habituation trials (Table 2) Thus, two groups received habituation trials in a "beak down" position one with CW and the other with CCW rotation. The remaining two groups were habituated with "beaks up one during CW and the other during CCW rotation. The habituation series for all birds comprised 15 accelerations of 10 /sec² for 12 sec each acceleration was followed by 1 min at constant velocity and a subthreshold deceleration (0 15 /sec² for 800 sec) Two pre and two post-tests were conducted immediately before and immediately after the habituation series. Within each group of birds the first pre and post-test rotations were conducted with "beak down for two birds (one *CW* the other CCW) and with beak up for the remaining two birds (one CW the other CCW) The second pre and post-test were in the same direction of rotation as the first for a given bird, but the animal's position was changed by 180 Pre- and post-tests comprised accelerations and decelerations of 10 /sec² separated by 2 min of constant velocity Thus, during pre- and post tests both directions of nystagmus were elicited (one direction during acceleration, the other during deceleration) for the two head and body positions ("beak down

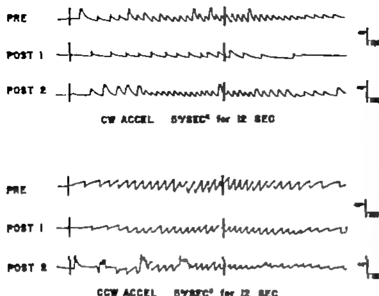


Fig 1 Tracings obtained from an African parrot. Vertical bars demarcate the stimulus periods; calibration markers appear to the right of the tracings. Upward deflections of the recording pen signify eye movements to the right; downward deflections signify eye movements to the left. A series of 15 unidirectional habituation trials occurred between the Pre- and Post 1 tests; Post-2 tests were given 2 weeks later A clear, directionally specific reduction is evident in the Post 1 tracings (CW accel), with a strong recovery from habituation apparent in the Post-2 data.

and beak up") During the habituation series, however only one direction of nystagmus was permitted to occur and the head and body were maintained in a single position.

Scoring

Measurements were made of slow phase eye displacement, frequency of nystagmus, and duration of response as outlined elsewhere (Mertens & Collins, 1967) For the African arrots, it was possible to convert the horizon slow phase measurements to degrees of eye vement by means of a calibration factor obtained from the birds responses to optokinetic stimulation (Capps & Collins, 1965) Vertical eye movements could be obtained only occasionally from a few of the dwarf parrots exposed to the optokinetic stimulator hence slow phase measurements of their vertical eye movements were expressed in arbitrary units.

RESULTS AND DISCUSSION

Stimulation of the lateral canals

A sample of the tracings of horizontal eye movements obtained from one of the African parrots appears in Fig. 1 Mean response scores for the six birds for measures of slow phase displacement, frequency and duration of nystagmus from the right eye are presented in Table 3 and depicted in Fig. 2.

For the direction of nystagmus repeatedly elicited during habituation trials, there was a decline for all three measures (relative change scores) from the Pre-test to Post 1 the decline was statistically significant for slow-phase ($t=5.38$ $p<0.01$) and frequency ($t=11.47$ $p<0.001$) measures, but not for duration ($t=1.36$ $p<0.05$ level $=2.57$) The unhabituated direction of nystagmus showed some decline from the Pre-test to Post 1 (except for an increase in the average duration score) but the declines were not significant Post 2 tests, conducted two weeks later showed recovery of the habituated response. Nystagmus in the unhabituated direction was approximately at the same level in the Post 1 and Post 2 sessions, but the latter tested as significantly lower than the Pre-test ($t=4.40$ $p<0.01$) due to the fact that all six parrots showed some decline from the Pre test to Post 2 (Table 4)

Comparisons among relative change scores appear in Table 5 The declines of slow-phase eye movement and frequency of nystagmus in the habituated direction from the Pre-test to Post 1 were significantly greater than both the Pre- to Post 1 change for the unhabituated direction, and the Pre to Post 2 change for

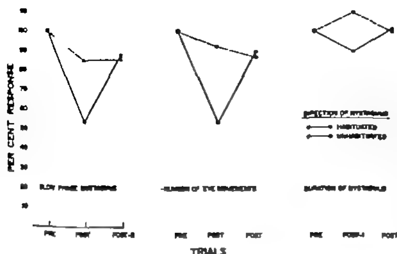


Fig. 2. Mean change (in per cent) of Post 1 and Post 2 scores from Pre-test levels for three measures of horizontal nystagmus obtained from African parrots. Pre-scores for the habituated and the unhabituated directions were each set at 100. Directionally specific effects of the habituation series (Post 1) are marked, and recovery (Post 2) is clearly evident.

the habituated direction. No other comparisons produced statistically significant differences.

Thus, ocular nystagmus from parrots underwent relatively rapid habituation and the response decline was (relatively) directionally specific. Considerable recovery occurred following a 2 week rest. These results are similar to those obtained from other animals (e g., Collins & Updegraff, 1966)

Stimulation of the vertical canals

Tracings of the vertical component of eye movements recorded from one eye of a dwarf parrot appear in Fig. 3 Mean response scores for duration, frequency and slow-phase eye displacement, for each of the two head positions, appear in Table 6 and are graphically presented in Fig. 4

Statistical analyses (Table 7) of Pre and Post-test scores indicate that nystagmus in the direction repeatedly elicited during habituation trials declined significantly for all three response measures when the birds were maintained in the same position as that imposed during the habituation series. For a change in position of 180 however only frequency of

Table 3. *Mean horizontal slow-phase eye displacement number of nystagmic beats, and response duration obtained from six African parrots during tests before and after a series of 15 unidirectional angular accelerations*

Data represent an average of two trials per subject (CW accel and CCW decel, or CW decel and CCW accel). The lateral canals were in the plane of rotation. All responses were recorded from the right eye of each bird. Pre- and Post-1 tests, respectively were given immediately before and after the habituation series, Post 2 tests were conducted 2 weeks later

Test	Slow-phase		Frequency		Duration	
	Degrees	of Pre	Beats	% of Pre	Seconds	of Pre
Habituated direction						
Pre	469 3		35.6		25 8	
Post-1	253 7	54 1	19.2	53.9	23 1	89 5
Post-2	410 5	87.5	31.9	89.6	26.1	101.2
Unhabituated direction						
Pre-	472 3		36.7		25.8	
Post 1	404 1	85.6	33.8	92.1	28.3	109 7
Post-2	405 6	85.9	32.1	87.5	25.8	100 0

Table 4 *Results of t tests between Pre-test and Post-test scores for the habituated and the unhabituated direction of nystagmus*

Measure	Comparisons	Direction of nystagmus Habituated	Unhabituated
Slow-phase	Pre vs. Post 1	5.38	1 76
	Pre -vs. Post 2	2.33	4 40
	Post 1 vs. Post-2	3.35	0.04
Frequency	Pre- vs. Post 2	11.47	2.43
	Pre vs. Post-2	0.70	2.21
	Post-1 vs. Post-	3.90	0.66
Duration	Pre- vs. Post 1	1.36	1.98
	Pre vs. Post 2	0.61	0.53
	Post-1 vs. Post 2	1.36	1 74

Levels of significance: 0 05 0.01 0.001

Table 5 *Result of t tests comparing the relative change in Post-1 and Post 2 responces from those of the Pre-test for the habituated and the unhabituated directions of nystagmus*

Comparisons of relative change	Measure	t
Habituated Pre- to Post-1 vs. Habituated. Pre to Post 2	Slow-phase	3.38
	Frequency	3.70
	Duration	1.38
Habituated. Pre- to Post 2 vs. Unhabituated. Pre to Post 1	Slow-phase	4.18
	Frequency	10.46
	Duration	1.93
Unhabituated. Pre to Post 1 vs. Unhabituated. Pre to Post 2	Slow-phase	1.02
	Frequency	0.60
	Duration	1 71
Unhabituated. Pre to Post 2 vs. ...ituated Pre- to Post-2	Slow-phase	0.36
	Frequency	0.72
	Duration	0.95

evels of significance: 0.05 *- 0.01 **- 0.001

Table 6. *Mean vertical slow-phase eye displacement number of nystagmic beats and response duration during pre- and post-habituation trials for 16 dwarf parrots*

Data for both the habituated and unhabituated directions of nystagmus are presented for the head position used during the 15 habituation trials and for a 180° change in head position ("unhabituated head position")

Measure	Test	Habituated head position, direction of nystagmus: Habituated	Unhabituated	Unhabituated head position, direction of nystagmus: Habituated	Unhabituated
Slow-phase (Arbitrary Units)	Pre	148.0	172.3	165.0	160.6
	Post	99 4	181 4	227 7	162.0
Number of Beats	Pre	34 1	32.2	34.3	27 7
	Post	17.5	28.8	26.9	26.3
Duration (Seconds)	Pre	23.1	22.1	22.5	21 4
	Post	18.0	22.1	21 1	20.4

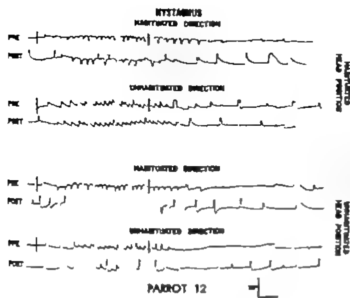


Fig 3 Tracings of ertical ny tagmus obtained from dwarf parrot. An optokinetic calibration was obtained for this bird. Vertical bars demarcate stimlus periods f 10°/sec² for 15 sec. Upward and downward deflections of the recording pen signify ertical ey movements in those same directions. A series of 15 unidirectional habituation trials occurred between the Pre- and Post-tests. Nystagmus declined significantly for the head position used and for the direction repeatedly elicited dur ing the habituation series. (Note the early secondary ystagmus in the topmost Post-tracing.) That same direction of nystagmus is considerably enhanced during the Post-test trial for the unhabituated head position.

nystagmus showed a significant Pre to Post test decline (Fig. 4 and Table 7)

Statistical comparisons of the relative change of Post-test scores from those of the Pre-test appear in Table 8 The decline in nystagmic output for the habituated direction and the head-body position used during the habituation series was significantly greater (0 05–0 01 levels) than the Pre to Post-test changes for either direction of nystagmus in the unhabitu ated head position, as well as for the unpract iced direction of nystagmus in the habituated head-body position. No other comparisons yielded statistically significant differences (Table 8 and Fig. 4)

Thus, habituation of vertical ocular nystagmus occurred in the birds and was confined to the direction repeatedly elicited. Moreover the reduction in response was relatively specific to the position maintained by the animals during the habituation series, i.e there was relatively little or no transfer of habituation when the same canals were stimulated but with the position of the animals changed by 180

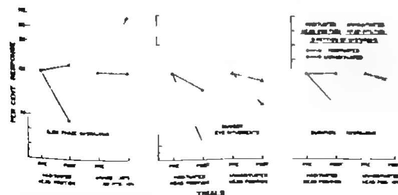


Fig 4 Mean change (in per cent) of Post-test scores from Pre-test levels for three measures of vertical nystagmus obtained from dwarf parrots. Pre-scores for the habituated and the unhabituated directio of nystagmus were each set at 100 Response reduction is directionally specific and relatively specific to the head position used during the habitua series.

Table 7 *Results of* t *tests between Pre- and Post test scores for the two directions of nystagmus and the two head-body positions*

Condition		*t*		
Direction of nystagmus	Head position	Slow-phase	Frequency	Duration
Habituated	Habituated	2.72	4.94	3.08
Habituated	Unhabituated	1 67	.58	0.98
Unhabituated	Habituated	0 41	1.60	0 00
Unhabituated	Unhabituated	0 08	1.05	1.31

Levels of significance: 0.05 0.01 0.001

These data agree with related results obtained from cats (Collins, 1969) and do not appear to be due to arousal factors caused by changing the position of the animals. In addition to the auditory stimuli used to help maintain arousal during response periods, several birds were given six or more additional trials in the "unpracticed" head–body position the response declined in the usual fashion. The birds were then placed in the opposite position (that maintained during the original habituation series) no recovery of response from the Post I levels was apparent. Further trials involved alternating the position of the animals No discernible effects of the handling occurred.

If arousal is excluded, the results permit two [illegible]ons (1) The specificity of the re[illegible] reduction is due to factors generally associated with the habituation procedure, i e., the animal becomes habituated to the general environment as well as to the acceleration stimulus, and significant" changes in the environment (including body position) result in a failure of habituation to transfer to the new situation. (2) The neural patterns generated by the semicircular canals, the otoliths, and other gravi-receptors interact to a considerable degree in determining habituation of nystagmus in some animals. This proposed interaction would thus be more dynamic than the previously reported regulatory effects (i.e., inhibiting or enhancing responses) of the otoliths on semicircular canal activity

ACKNOWLEDGMENTS

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The animals used for this experiment were lawfully acquired and treated in accordance with the "Principles of Laboratory Animal Care issued by the Animal Facilities Standards Committee of the Animal Care Panel, United States Department of Health, Education, and Welfare, Public Health Service, March 1963

RESUME

Le nystagmus oculaire horizontal chez des oiseaux, dont le mouvement fut restreint, fut habitué dans une manière spécifique à un côté de réponse en exposant les oiseaux aux accélérations angulaires répétées. De rétablissement assez grand de réponse fut évident après deux semaines sans stimulation. Le

Table 8. *Results of* t *tests on relative change scores (Pre- to Post test) between the various combinations of head–body position and direction of nystagmus (Hab = Habituated Unhab = Unhabituated)*

The Pre- to Post-test decline for the habituated direction of nystagmus in the habituated head position was significantly greater than the Pre- to Post-test changes obtained for other head-position and nystagmus-direction combinations

Comparisons					*t*		
Head position	Nystagmus direction	vs.	Head position	Nystagmus direction	Slow phase	Number of beats	Duration
Hab	Hab	vs.	Hab	Unhab	3.42	3 94	2.56
Hab	Hab	vs.	Unhab	Unhab	2.34	5.47	1.53
Hab	Hab	vs.	Unhab	Hab	3.26	3 10	1.51
Hab	Unhab	vs.	Unhab	Hab	0.69	1 13	1 10
Hab	Unhab	vs.	Unhab	Unhab	0.46	0.53	1 44
Unhab	Unhab	vs.	Unhab	Hab	1.35	0.90	0.05

Levels of significance: 0.05 0.01

nystagmus vertical fut habitué d'une manière semblable chez un autre groupe d'oiseaux qui furent placés en position avec les canaux verticaux au plan de rotation. En changeant de 180° la position de ces oiseaux après les épreuves d'adaptation, on put stimuler les mêmes canaux verticaux, mais avec les otolithes et d'autres récepteurs de gravité situés dans une manière différente qu'auparavant. L'adaptation fut *relativement spécifique au côté* souvent provoqué de nystagmus et à la position de la tête et du corps tenue pendant les épreuves d'adaptation. Une interaction dynamique entre les récepteurs de gravité et les canaux semi-circulaires fut proposée comme trait possible d'adaptation nystagmique.

ZUSAMMENFASSUNG

An Vögeln, dessen die Bewegung zurückgehalten wurde, wurde der okulare laterale Nystagmus infolge wiederholter angulärer Beschleunigungen richtungsspezifisch gewohnt. Wirkliche Reaktionswiedergewinnung kommt deutlich nach zwei Wochen ohne Reizung vor. An einer anderen Gruppe von Vögeln, mit den vertikalen Bogengängen im Beschleunigungsebene, wurde der vertikale Nystagmus ähnlich gewohnt. Beim verändern 180° die Lage dieser Vögel nach den Gewöhnungsprüfungen, konnte mann die selbe vertikalen Bogengangen reizen, aber mit den Otolithen und andere Empfänger der Schwere anders als früher orientiert. Die Gewöhnung war verhältnismässig spezifisch für die Nystagmusrichtung die mann wiederholt hervorgerufen hat und für die Kopf und Körperlage die während der Gewöhnungsprüfungen erhalten wurden. Eine dynamische Wechselwirkung zwischen Empfänger der Schwere und die Bogengänge wurde wie mögliche Eigenschaft nystagmischer Gewöhnung vorgeschlagen.

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THE DIAGNOSTIC VALUE OF GELLE'S TEST

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A method is described for the quantitative performance of the test of Gellé, the tone threshold for air as well as bone-conduction as a function of air pressure in the auditory canal being determined by amplitude modulation. Special attention is paid to the influence of the occlusion effect and the necessity for masking in bone-conduction measurements.

The author has tried to determine the range in which the results may vary for normals as well as for patients with otosclerosis. In agreement with many other investigators, it has been found that in cases of stapedial ankylosis the bone-conduction threshold is not affected by air-pressure changes in the auditory canal. An attempt is made to explain this phenomenon.

In 1881 Gellé described the following test: hen the pressure in the external auditory is increased by means of a Politzer bal-)a loudness decrease should be found for both air-conduction and bone-conduction in individuals with normal hearing (positive Gellé) In the presence of stapedial ankylosis, this phenomenon occurs for air-conduction but not for bone-conduction (negative Gellé)

Gellé sought the explanation of this phenomenon in the increased labyrinthine pressure Fixation of the stapes would prevent transfer of the increased pressure from the tympanic membrane to the inner ear He therefore considered the test to provide a diagnostic criterion for otosclerosis (1885)

Most of Gellé s contemporaries, including Bezold (1887) Politzer (1893) and Bing (1899) agreed with Gellé about the localization of the pressure increase, but Bartsch (1885) held the opinion that increased tension in the tympanic membrane and ossicular chain caused the reduced hearing. He furthermore stated that this effect also occurred at negative pressure in the auditory canal.

In 1921 Griessmann constructed an otosclerometer" permitting quantitative determination of the "Gellé effect" He found no difference between the results in stapedial ankylosis and other forms of ossicular chain fixation. Van Dishoeck (1937) carried this principle further in his pneumophone In combination with the audiometer which had meanwhile come into general use, various investigators have performed quantitative studies, including Aubry & Giraud (1943) Thullen (1952, 1954), Kietz & Zangemeister (1953), Dudok de Wit & van Dishoeck (1959) Mehmke (1960) Huizing Jr (1960) Old *et al.* (1961 1962) and Arnold & Schindler (1963)

In general, these investigators have come to similar conclusions, i.e. that both positive and negative pressure in the middle-ear or auditory canal leads to the reduction of hearing acuity and that the effect is more pronounced for the low than for the high frequencies, with a maximum in the region of 250 to 1000 Hz. The marked spread in the results and the inaccuracy still inherent in the test in spite of various refinements, however have tended to prevent its application for the individual diagnosis of middle-ear defects.

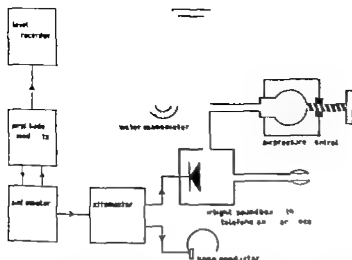


Fig. 1. Diagram of the apparatus.

In the present study an attempt was made to evaluate the spread of the results of the test, both in the individual and within groups of subjects with normal hearing and of patients with stapedial fixation due to otosclerosis.

APPARATUS AND METHOD

The relation between air-pressure in the outer ear and the hearing threshold was determined by administering a number of fixed air-pressures in a given sequence to the auditory canal, while the subject himself changed the intensity of the stimulus by means of amplitude modulation (Békésy technique). The apparatus (Fig. 1) consisted of a Peekel audiometer type D 4 connected to an amplitude modulator and a Bruel and Kjaer level recorder type 2305. The modulation range of the combination was 50 dB, the modulation of rate 4 dB/sec. The telephone was enclosed in an airtight box, which was connected via a rubber tube with the auditory canal of the ear to be tested and with a water-manometer and a pressure regulator. In this way any air pressure up to 100 cm water pressure, both positively and negatively could be obtained in the auditory canal, for air-conduction as well as for bone-conduction measurement, in which case the bone-conduction receiver was placed on the mastoid.

The study was performed in two groups of subjects, a group of twelve young persons with normal hearing and a group of twenty five patients with *conduction* deafness due to stapedial fixation in otosclerosis. In the second group, a bone-conduction loss of less than 20 dB between 500 and 2000 Hz was considered normal (Carhart notch). In all cases the diagnosis was surgically confirmed after the test.

All measurements were made in a sound-proof room. The hearing threshold was measured at air-pressures of 0, −10, −20, −40, −80, 0, +10, +20, +40, +80 and again 0 cm H_2O for both air- and bone-conduction. The sound stimulus consisted of a continuous pure tone of 500 Hz. The choice of this frequency was based on the findings of earlier investigations indicating that the maximum obtainable effect occurs in the frequency range of 250 to 1000 Hz.

All thresholds were determined compared with a *sensational* level of 0 dB at an air pressure in the auditory canal of 0 cm H_2O. Each threshold measurement consisted of a cycle of 10 periods, permitting calculation of *the threshold to an accuracy of about 1 dB*. The outer limits of the amplitude variations have been found to lie 4 to 6 dB apart for a trained subject and almost 10 dB apart for an untrained subject or patient, both for a modulation rate of 4 dB/sec.

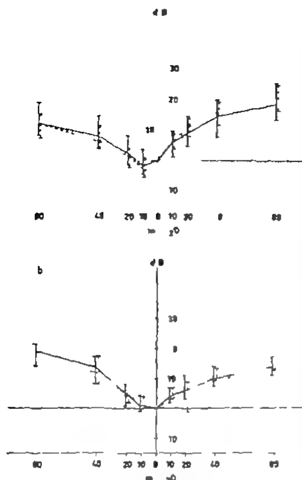


Fig 2 (a) Air and bone-conduction threshold as a function of air-pressure in the auditory canal, series of ten measurements in one normal hearing subject. — air-conduction bone-conduction. *(b)* Air and bone-conduction threshold as a function of air pressure in the auditory canal series of ten measurements in another normal hearing subject. — air-conduction, bone-conduction.

EXPERIMENTS

In a number of pilot studies we tried to measure some side-effects that might possibly influence the test procedure or the results. The normal fluctuations in hearing acuity during the time required for the test (30–45 min) apart from the effects of air-pressure change were determined by administering the complete test procedure to several well-trained subjects, while the air pressure in the auditory canal was kept constant. This was done for both air and bone-conduction at pressures of 0 +40 and −40 cm H_2O We found threshold fluctuations of up to about 4 dB and, if kept constant, neither positive nor negative pressure was found to have any influence. These results are in agreement with the findings of von Békésy (1947) who found variations of about 5 dB

To establish whether the sequence of the pressure changes could influence the results of the test, all the pressure values used were offered in different sequences to one subject, five times each. The twenty five threshold values thus obtained were arranged in a so-called Latin Square. The result showed no influence of a given sequence.

To determine the spread in results in persons with normal hearing (both in one individual and for a group) the following measurements were done. First, two young, well-trained subjects with normal hearing were each given the complete test ten times over a fourteen-day period. For both air and bone-conduction, both subjects showed an individual variation of about 10 dB (Fig. 2) Then a group of ten persons with normal hearing were given the test individually The mean values of the results in this group agreed well with those of the preceding test, but the spread was greater varying from 5 to 15 dB (Fig. 3) The effect of positive and negative pressure was in the absolute sense virtually the same

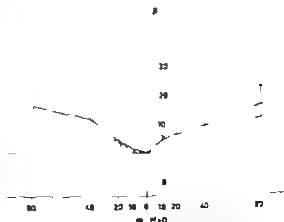


Fig 3 Air and bone-conduction threshold as a function of air-pressure in the auditory canal in ten normal hearing subjects. — air-conduction bone-conduction.

It is remarkable that both in the two series of repetitions with one subject and in the test series in ten other subjects, all with normal hearing, the bone- as well as the air-conduction showed about the same changes *quantitatively* whether the air-pressure in the auditory canal increased in negative or positive value. This observation is in conflict with those of other investigators. Van Dishoeck (1940) reported for air-conduction at 250–500 Hz maximal threshold increases of 25–30 dB and for bone-conduction 10–15 dB. These findings were confirmed by Thullen (1952), Dudok de Wit & van Dishoeck (1959), Mehmke (1960), Huizing Jr (1960), and Arnold & Schmidler (1963).

The explanation of these conflicting results should perhaps be sought in the fact that none of these authors masked the opposite ear during bone-conduction measurements. It has long been known (Bárány 1940, Carhart, 1950) that for a bone-conduction threshold difference of a few decibels to the disadvantage of the ear being tested, the tone is perceived in the other cochlea. Such a threshold difference may develop at a pressure difference in the auditory canal amounting to a few centimetres water pressure. It is also necessary to take into account the occlusion effect, a phenomenon by which the sound threshold for bone-conduction drops when the auditory canal is obstructed. Huizing Jr (1960) demonstrated that the maximum threshold reduction occurs in occlusion at ½ λ or a multiple thereof.

With the airtight closure of the auditory canal required for the Gellé test, the occlusion effect cannot be eliminated, but it remains constant throughout the test if the length of the air-column does not vary, even when the height of the meniscus of the water-manometer is changed. In our experimental set-up the acoustical length of this column was about 36 cm. For 500 Hz, the half wave length is 33 cm, and therefore the occlusion effect may thus be considered as virtually maximal. A series of experiments done to elucidate this point showed a difference of 17 to 18 dB between the conduction thresholds with and without the sound-tube in the auditory canal. It is obvious that occlusion and air-pressure change in the auditory canal have an opposite effect on the bone-conduction threshold. Due to the first phenomenon the threshold drops and in a person with normal hearing the tone is definitely perceived in the ear being tested. If the pressure in the auditory canal nevertheless rises to a certain height, the occlusion effect is more than compensated for and cross-over hearing can occur.

This hypothesis explains not only the differences between bone- and air-conduction but also the maximum threshold increase of about 15 dB for the bone Gellé found by all other authors in individuals with normal hearing. This value is in good quantitative agreement with the "threshold advantage" that the occlusion effect gives the ear being tested. Huizing Jr (1960), who used a method in which he tried to exclude the occlusion effect, provides the only exception among the published results. The bone-conduction threshold increases observed at 500 Hz in his experiments are lower than those of the other authors.

On the basis of these considerations, for all subjects who were to receive the stimulus via bone-conduction in our experiments, the contra-lateral ear was masked with a low frequency noise. The bone-conduction threshold of the ear being tested was determined with the Gellé tube in the ear. After this, the contra-lateral ear was masked up to the highest possible intensity at which no over-masking occurred. Since the threshold of the tested ear rises due to the change in pressure, the risk of over-masking in the course of the test becomes steadily smaller, while the margin between the test tone and the masking noise remains large enough to exclude stimulation of the non-tested cochlea by the test tone. With this method it was not necessary to change the noise-intensity during the test, which might have introduced a source of error. When necessary, the same masking procedure was applied to the patients in the study.

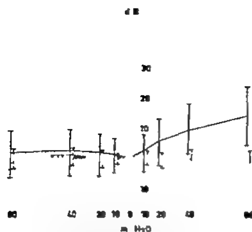


Fig 4 Air- and bone-conduction threshold as a function of air-pressure in the auditory canal in 25 patients with otosclerosis. —, air-conduction, ---- bone conduction.

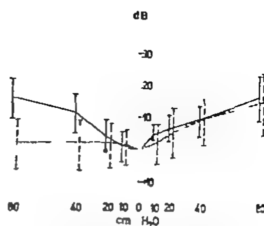


Fig 5 Air-conduction threshold as a function of air pressure in the auditory canal. Ten normal hearing subjects compared with 25 cases of otosclerosis. — normal, ---- otosclerosis.

RESULTS

With respect to the air-conduction results in otosclerosis (Fig. 4) it is striking that the effect is distinctly greater for positive than for negative pressures, in contrast to the findings in subjects with normal hearing, in whom no essential difference was found on this point. The variation range for the same pressures is about the same for both groups, and varies between 5 and 15 dB Generally the data ·· for positive pressure in otosclerosis pa- ·· and normal subjects were about the same For negative pressure, the overlapping is smaller but nonetheless distinctly present. On the basis of the air-conduction findings, no difference can be distinguished between the normal situation and otosclerosis (Fig. 5)

The absence of a significant difference between the air-conduction results for normals and cases of stapedial ankylosis throws even more emphasis on the difference in the results of the bone-conduction measurements. In normals, the "Gellé effect" on bone-conduction is qualitatively and quantitatively the same as on air-conduction, but in cases of stapedial fixation the bone-conduction effect is entirely absent. In all patients with otosclerosis the bone

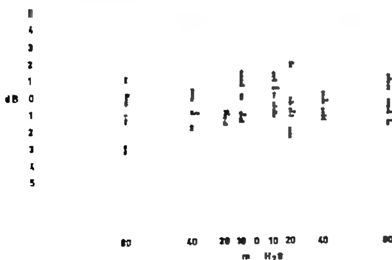


Fig 6. Threshold levels for bone-conduction as a function of air pressure in the auditory canal in 25 patients with otosclerosis.

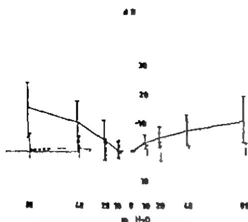


Fig. 7. Bone-conduction threshold as function of air-pressure in the auditory canal; ten normal hearing subjects compared to 25 cases of otosclerosis. —, normal; otosclerosis.

conduction threshold does not change more than 5 dB even for the highest pressures applied (+ 80 and − 80 cm H_2O), and neither the variation range nor the means show a significant difference between the various series of values measured for a given pressure. The range of variation is furthermore distinctly smaller than that of the normals, and lies in the same order of magnitude as the threshold variations measured without pressure (Békésy 1947).

Fig. 6 shows the data recorded in 25 otosclerosis patients. About 80% lie within 2 dB of the zero line.

Although the bone-Gellé performed according to the above-described method permits a distinct differentiation between a normal middle-ear and one suspected of stapedial fixation, the test is not pathognomonic for otosclerosis. In two patients with a traumatically interrupted ossicular chain and in three with adhesive otitis media, a completely negative bone-Gellé was also obtained. From these findings it may be concluded that, on the basis of the bone-Gellé for an air-pressure difference of more than 40 cm water between the middle-ear and the external auditory canal, a distinction can be made in individual cases between a normal and an anomalous middle-ear but that a closer typification of the anomaly is not possible (Fig. 7).

DISCUSSION

The results of the tests performed in this study confirm the findings of many previous investigators, i.e. that a pressure change in the auditory canal of patients with otosclerosis influences only the air-conduction threshold, not the bone-conduction threshold. The latter i not difficult to understand. The stapes is fixed impeding not only the transfer of vibrations from the middle-ear to the inner ear but also the influence on the cochlea of middle-ear impedance changes. Seen in this light, however it is not directly clear why the tone threshold of a stimulus applied via the auditory canal is indeed influenced by air pressure variations. In our opinion, this phenomenon might be explained as follows.

When the air-Gellé is applied to an ear with stapedial ankylosis, the sound-pressure in the auditory canal amounts, depending on the size of the air–bone gap, to 30 to 50 dB more than would be the case in a normally functioning middle-ear. When there is no difference in air pressure between the middle-ear and the auditory canal, this sound-pressure will be sufficient to stimulate the cochlea by the round window since it is not seriously impeded by the tympanic membrane. However air-pressure change in the auditory canal will stretch the drum membrane, thus increasing the reflection of sound and decreasing the sound-pressure in the tympanic cavity.

The change in the tone threshold due to air pressure changes in the external auditory canal thus originates via a middle-ear with stapedial ankylosis in a different way than via a normal middle-ear. In the latter case the pressure change disturbs the optimal adjustment of the middle-ear to the cochlea, and the ossicular chain is impeded in fulfilling its function of transmission. In the former case the cochlea is stimulated without participation of y ossicles and the tympanic

no function but *(to the contrary)* forms an impedance whose degree is determined by the position and tension of the membrane

CONCLUSION

The Gellé test, with the modifications still applied today (including the method used in this study) has no value for the individual diagnosis of anomalies of the middle-ear The variation range of the measured data is too large and the difference between the results obtained in subjects with normal hearing and in patients with a conduction defect are too small to permit reliable conclusions in an individual case When sufficient accuracy can be achieved in the test, the investigation of groups of patients and the statistical analysis of the material can, however give a better insight into the function of the middle-ear and can therefore contribute to a better theoretical basis for the surgical treatment of middle-ear defects.

ZUSAMMENFASSUNG

Es wird eine Methode zur quantitativen Ausführung des Gellé-tests beschrieben, wobei für verschiedene Luftdruckwerte im Gehörgang die Tonschwelle, sowohl für Luftleitung als für Knochenleitung, mit Hilf der Amplitudenmodulation bestimmt wird. Mit Nachdrücklichkeit wird auf den Einfluss des Okklusionseffektes hingewiesen und auf die Unerlässlichkeit von Maskierung bei den Messungen der Knochenleitung. Der Autor hat versucht die Reichweite zu bestimmen innerhalb welcher die Resultate variieren konnen, dies geschah bei Normalhorenden und bei Otosklerosepatienten. Wie schon viele andere Forscher festgestellt haben erwies es sich, dass bei Patienten mit einer Stapesf xierung die Knochenleitung nicht durch Luftdruckveranderungen im Gehorgang beeinflusst wurde Es wi d versucht dieses Phänomen zu erklären.

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HISTOLOGIE DE LA SURDI MUTITÉ HÉRÉDITAIRE RÉCESSIVE

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Reçu Septembre 1 1969

Les auteurs présentent le cas d'un sourd-muet héréditaire de la tribu d'Ayent, décédé accidentellement. L'examen clinique avait montré un reste d'ouïe peu important mais assez étendu, la perception allant de 250 à 2 000 cs, l'examen histologique révéla une absence totale de l'organe de Corti avec altération importante de la partie inférieure du labyrinthe tandis que l'utricule et les canaux semi-circulaires étaient normaux.

Il y a longtemps qu'on s'intéresse au problème de la surdité héréditaire et aux altérations histologiques qui sont à la base de l'affection. On parle aujourd'hui encore du type Mondini et du type Michel d'après deux cas décrits par ces auteurs en 1791 et en 1863 Et pourtant l'histologiste le plus averti est actuellement encore parfaitement incapable, au vu du simple examen histologique, de dire si une surdité est d'origine héréditaire ou acquise Le problème est donc toujours d'un brûlant intérêt et toute nouvelle pierre apportée au dossier peut nous apprendre quelque chose

Différents auteurs ont cherché à mettre de l'ordre dans la question et à établir une classification. Ormerod (1960) distingue trois grandes classes d'altérations

1 Il peut y avoir un défaut de développement de l'organe auditif ou d'une de ses parties (Failure to develop)
2 Une interruption, un arrêt dans un moment quelconque de la formation de l'organe auditif et
3 Une dégénérescence de parties de l'organe auditif qui s'étaient développées normalement jusque là.

Il peut en résulter différents types qu'on a baptisés selon le nom des auteurs qui les ont décrits les premiers.

1 Type Michel. aplasie totale.
2 Type Mondini–Alexander La cochlée est représentée par un simple tube incurvé. Le vestibule et les canaux semicirculaires sont profondément altérés. Le labyrinthe osseux est touché aussi bien que le membraneux.
3 Type Bing–Siebenmann. Le labyrinthe osseux est normalement formé mais les parties membraneuses sont sous-développées. L'altération atteint aussi bien le labyrinthe supérieur que l'inférieur
4 Type Scheibe: La malformation est limitée à la cochlée membraneuse et au saccule
5 Type Siebenmann: Altérations de l'oreille moyenne (surdité endémique crétinisme)
6 Microtie et altération du canal auditif externe

Cette classification est cependant fort loin de résoudre tous les problèmes. On admet en général que la surdité héréditaire récessive correspond au type Scheibe Il n'y aurait donc pas d'altération du squelette osseux, la partie supérieure du labyrinthe serait entièrement normale Par contre la partie inférieure présente une atrophie ou même une disparition totale de l'organe de Corti, un défaut de développement de la membrane tectoriale qui est repliée sur elle-même ou absente un collapse de la membrane de Reissner qui peut même être adhérente à la strie vasculaire parfo

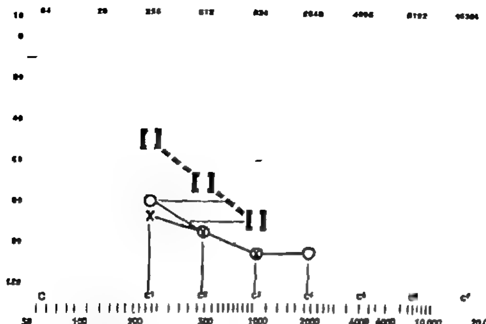


Fig 1 La surdité est très grave le reste d'ouïe est cependant relativement important puisque la perception s'étend de 250 à 2 000 c/s. Les courbes de conduction osseuse sont vraisemblablement dues à une simple perception tactile.

cependant il y aurait au contraire une ectasie du canal cochléaire avec dilatation de la membrane de Reissner La strie vasculaire est parfois atrophiée, parfois elle présente des proliférations avec accumulation de concrétions, ces concrétions sont PAS positives. On peut, dans même oreille, constater à certains endroits collapse de la membrane de Reissner et à une ectasie du canal cochléaire Les altérations atteignent toujours la spire de base, elles peuvent être moins accentuées sur le second ou le troisième tour de spire, elles peuvent atteindre aussi la cochlée tout entière. Enfin, on constate en général une atrophie de la macule sacculaire, du ganglion spiral et des fibres nerveuses correspondantes. Il s agit donc vraisemblablement d une régression d'organes primitivement normaux.

Ce tableau paraît net et bien précis, cependant, on a pu constater des altérations pratiquement identiques lors de surdimutités certainement acquises, par exemple après rubéole de la mère pendant la grossesse (Nager 1952) C'est pour cette raison que Altmann (1964) considère que la régression peut être soit spontanée, et il s agit alors d'une affection héréditaire, soit secondaire à un facteur extrinsèque (infection virale, hémorragie etc.) et il s agit alors d'une surdité acquise.

Nous ne disposons cependant d aucun critère histologique permettant de différencier les deux étiologies.

La question se complique encore du fait qu on ne possède actuellement que fort peu de cas de surdi-mutité certainement héréditaire qui aient été examinés à la fois cliniquement et histologiquement.

D après Ward, Kinney et Lindsay on ne connaissait en 1962 aucun cas examiné histologiquement qui ait eu un examen clinique avec audiogramme En 1963 seulement, Buch & Jorgensen ont publié un cas pratiquement complet. Il s agissait d'une femme âgée de 37 ans dont une soeur était également sourde-muette L examen clinique avait montré une perception des basses fréquences des deux côtés à 80 dB Les réactions labyrinthiques thermiques étaient normales, l'encéphalographie gazeuse montrait une légère atrophie de la région frontale Les radiographies du crâne



Fig. 2. Oreille gauche anomalies de l'oreille moyenne. *V.* vestibule. *F.* facial. *S.* étrier mal formé. *E.* enclume, longue apophyse.



Fig. 3. Le canal cochléaire est fortement collabé, l'organe de Corti est réduit à un simple épaississement cellulaire dans lequel on ne reconnaît aucune structure, le ganglion spiral est complètement atrophique. La strie vasculaire est fortement altérée, elle présente des proliférations avec hyperplasie et formation de véritables replis.

l'électroencéphalogramme étaient normaux, le Wassermann négatif. Le labyrinthe supérieur était normal, le saccule était en partie collabé dans la macule sacculaire, il y avait des dépôts PAS positifs, de même que dans la strie vasculaire. Le canal cochléaire était en partie ectasié. L'organe de Corti ne pouvait être identifié nulle part, la membrane tectoriale était enroulée sur elle-même, les fibres nerveuses de la lame spirale étaient réduites en nombre de même que les cellules du ganglion de Corti. Le nerf VIII présentait des signes de dégénérescence sous forme de fibrose interstitielle. Cependant, les fibres nerveuses conduisant aux ampoules des macules sacculaires et utriculaires étaient normales, de même que le ganglion vestibulaire. Il s'agissait donc vraisemblablement d'une surdité héréditaire récessive: il y avait un reste d'ouïe dans les fréquences inférieures et l'examen histologique montrait des altérations du type Scheibe.

Nos connaissances ont, d'autre part, passablement progressé grâce aux examens faits sur l'animal.

Nous connaissons des malformations héréditaires de l'oreille interne chez l'animal soit qu'il s'agisse de tares spontanées, soit de mutations obtenues artificiellement, par exemple par application de rayons X. Il y a, cependant, des différences importantes entre l'homme et l'animal. Chez l'animal, il y a toujours participation du labyrinthe supérieur. Il peut même arriver que le labyrinthe supérieur soit seul atteint, tandis que l'inférieur est normal. Souvent les altérations ne sont pas présentes à la



Fig 4 L organe de Corti est totalement absent, la membrane tectoriale repliée sur elle-même, la strie vasculaire très altérée présente de grosses proliférations.

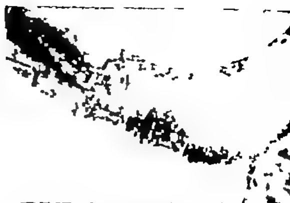


Fig 5 L organe de Corti est remplacé par un léger épaississement de la membrane basilaire la membrane tectoriale est enroulée sur elle-même, réduite à un simple moignon.

naissance, elles se développent plus tard, parfois seulement à la puberté Trualove (1956) par exemple, a étudié l'anatomie de la souris dansante (fidget mouse) les anomalies sont limitées aux canaux semi-circulaires et aux régions immédiatement adjacentes. Le canal semi-circulaire horizontal est absent, les autres sont à peine ébauchés, la structure histologique de la cochlée est, par contre, parfaitement normale, le saccule, l'utricule, le canal et le sac dolymphatiques sont normaux. Chez les sou- dansantes à queue courte, Bonnevie (1936) [illegible] l'absence des canaux semi-circulaires mais la cochlée est également altérée, présentant seulement une ébauche de courbe en spirale avec structure rappelant l'organe de Corti Fischer (1956) a étudié une race de souris dansantes (Dreher) Les examens histologiques ont montré les aspects les plus variables qui peuvent se ramener à deux groupes principaux Dans le premier Il y a augmentation de l endolymphe, le canal cochléaire est dilaté. au lieu du ductus reuniens, il y a une large communication entre la cochlée et le labyrinthe supérieur Saccule et utricule forment un espace commun, le canal sacculo-utriculaire manque, l organe de Corti est atrophié, les macules statiques n ont pas d'organe sensoriel Dans le second groupe, la membrane de Reissner est collabée, l organe de Corti est plus ou moins détruit. Ces animaux présentent aussi des malformations cérébrales d'après les uns, celles-ci sont primaires, elles sont la cause des altérations de l'oreille interne d après d'autres, elles sont seulement concomittantes, les deux malformations ayant une origine commune

Hertwig (1944) a obtenu par action des rayons X chez la souris des mutations provoquant une surdité récessive (Röntgenmutierte Kreisler Mäuse) le canal et le sac endolymphatiques manquent, les canaux semi-circulaires sont incomplètement développés, la pression endolymphatique est augmentée, le canal cochléaire consiste en une sorte de formation kystique herniée dans le méat auditif interne, altérant ainsi le développement du cerveau Quant à la pathogénie, Altmann fait remarquer que les altérations de l'organe de Corti ne doivent pas être secondaires à celles de la strie vasculaire puisqu on les trouve aussi parfois dans les macules qui ont une vascularisation et une nutrition indépendantes.

Les malformations constatées chez l'homme ne sont pas directement comparables à celles des rongeurs puisqu'il n y a pas d'altération du labyrinthe supérieur elles sont, par contre, analogues à celles trouvées chez le chat et le chien Il est possible que la valve de Bast joue ici un rôle empêchant le collapse de s'étendre au labyrinthe supérieur (Altmann). Chez



Fig. 6. La strie vasculaire présente des proliférations importantes, la structure normale est plus reconnaissable.



Fig. 7. L'utricule et les canaux semi-circulaires sont normaux. On reconnaît la macule utriculaire qui est normalement développée de même que la crête ampulaire. La macule sacculaire par contre est fortement modifiée. *U* macule utriculaire; *S* macule sacculaire; *CA* crête ampulaire.

l'animal comme chez l'homme les altérations acquises peuvent être exactement semblables aux malformations héréditaires.

En Suisse la surdi-mutité héréditaire a fait depuis longtemps l'objet de nombreuses études cela provient, en grande partie, de l'intérêt qu'y ont porté plusieurs hommes éminents: Siebenmann, Nager Ulrich et plus tard Hanhart (1938) dont les travaux sur le mode de transmission de la tare sont particulièrement importants cela provient aussi probablement du fait que la surdi-mutité est particulièrement fréquente chez nous et surtout que son étude est grandement facilitée parce que beaucoup de nos vallées sont restées longtemps isolées et retirées du monde Les habitants ont toujours continué à se marier entre eux, si bien que les tares ont persisté au cours des siècles. Hanhart (1938) a ainsi pu relever douze foyers de surdi-mutité récessive en Suisse

Le plus important d'entre eux est situé à Ayent en Valais, on a pu y dénombrer plus de 70 sourds-muets qui ont été examinés cliniquement par Ulrich, puis plus tard par l'un d'entre nous (Secrétan, 1954) La plupart d'entre eux ont été l'objet d'examens audiométriques, publiés en 1954 (Secrétan) L'un d'eux V.M. mourut accidentellement dans le courant de l'année 1966 Grâce à l'obligeance du Dr Pellissier de Sion, auquel nous adressons nos plus vifs remerciements, nous avons pu entrer en possession des rochers de ce patient.

V.M est né en 1899 Il fut élevé à l'Institut de sourds-muets de Géronde Cordonnier-paysan, il donnait l'impression d'une intelligence éveillée et présentait la voix typique d'un sourd-muet L'examen ORL ne montrait rien de particulier les tympans étaient calmes et bien mobiles au Siegle, l'audiogramme (voir Fig. 1) montrait un léger reste auditif s'étendant des fréquences 250 à . 000 cs, la perte allant de 80 à 105 db L'appareil vestibulaire était inexcitable aux excitations thermiques même avec l'eau à 10 on n'obtenait pas de réactions nystagmiques.

L'examen histologique des rochers a montré d'importantes altérations. A droite l'oreille moyenne est normale à gauche, par contre on constate une malformation des osselets (Fig 2) L'étrier est accolé au canal du facial, le col et la branche postérieure y sont adhérents l'enclume est ab ente, tandis que le

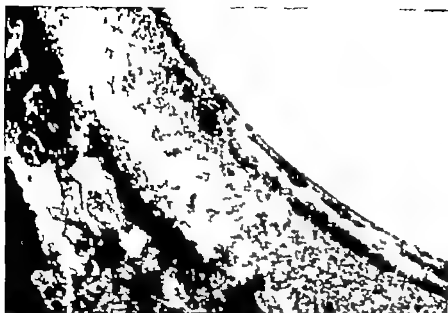


Fig 8 La macule sacculaire est profondément altérée. l'epithélium est formé de cellules allongées, platies ou même absent. Il y a, d'autre part, des formations kystiques.

est normalement constitué la longue branche de l enclume est remplacée par une fine apophyse osseuse qui s articule avec l'étrier

L oreille interne présente par contre un status identique des deux côtés: Le limaçon montre un canal cochléaire fortement collabé (Fig. 3) La membrane de Reissner est parfois même partiellement adhérente à la strie vasculaire. L organe de Corti a totalement disparu, il est remplacé par un simple épaississement de la membrane basilaire aucune structure n est plus reconnaissable La membrane tectoriale est enroulée sur elle-même réduite à un simple moignon (Fig. 4 et 5) La strie vasculaire présente des proliférations importantes, la structure normale n est plus reconnaissable, on ne distingue ni cellules chromophobes, ni cellules chromophiles (Fig. 6) Les cellules ganglionnaires ont, d'autre part, presque totalement disparu, il n en subsiste que quelques restes épars (Fig. 3)



Fig 9 Le canal endolymphatique montre une structure norma'e.

Labyrinthe postérieur les canaux semi-circulaires et l utricule sont parfaitement normaux (Fig. 7) la macule utriculaire n est pas altérée Le canal et le sac endolymphatiques ne montrent pas non plus de modifications pathologiques (Fig. 9 et 10) Le saccule est, par contre, profondément modifié (Fig. 7) Il est ectasié ses parois sont appliquées contre la cavité osseuse du vestibule l espace périlymphatique étant pratiquement absent. La macule sacculaire est fortement altéré, il n y a, ou bien pas d épithélium, ou bien seulement un épithélium formé de cellules allongées, aplaties on y trouve également des formes kystiques (Fig. 8) Les fibres nerveuses du nerf sacculaire ont



Fig. 10 Tracé normal du canal endolymphatique; l'os montre, par contre, quelques altérations, on constate un nombre augmenté d'îlots cartilagineux non ossifiés.

pratiquement disparu, tandis que les fibres du nerf utriculaire sont normales.

L'os montre, d'autre part, quelques altérations. On constate un grand nombre de globules osseux et un nombre augmenté d'îlots cartilagineux non ossifiés (Fig. 10).

En résumé, l'examen histologique montre de profondes altérations du labyrinthe inférieur et du saccule caractérisées par une disparition totale de l'organe de Corti, un collapse du canal cochléaire, des altérations importantes de la strie vasculaire, une atrophie de la macula sacculaire, une disparition presque totale des fibres des nerfs cochléaires et sacculaires; l'utricule et les canaux semi-circulaires sont normaux, de même que le canal et le sac endolymphatiques.

CONCLUSIONS

M. V. M., décédé accidentellement à l'âge de 67 ans était un sourd-muet héréditaire de la tribu d'Ayent. A part sa surdité, c'était un homme normalement conformé et intelligent. L'examen clinique avait révélé un reste d'ouïe peu important mais bilatéral s'étendant des fréquences 250 à 2 000 cs avec une perte oscillant entre 80 et 105 dB. Les épreuves thermiques révélaient une inexcitabilité vestibulaire totale même à l'eau à 10°.

L'examen histologique a montré qu'il s'agissait chez ce malade d'une malformation du type Scheibe; les altérations sont cependant identiques à celles constatées par Nager dans des cas de surdité acquise à la suite de rubéole.

Quoique l'examen histologique n'ait pas montré d'altérations de l'utricule et des canaux semi-circulaires, le malade n'avait présenté durant sa vie aucune réaction aux excitations thermiques, même l'injection d'eau à 10° restait sans aucun effet.

Il y avait d'autre part une malformation des osselets du côté gauche alors qu'à droite l'oreille moyenne était normale.

SUMMARY

The histology and clinical aspects of deaf-mute of the consanguinity of Ayent, who died accidentally is presented. The clinical examination revealed a slight remnant of hearing which, however, was spread between 250 and 2000 cs. Histologically the organ of Corti was completely missing. Important alterations of the inferior labyrinth were also present, whereas the utriculus and semicircular canals were normal.

ZUSAMMENFASSUNG

Histologie und Klinik eines Taubstummen aus der Sippe von Ayent, der an einem Unfall gestorben ist, werden dargelegt. Klinisch fand sich ein kleiner rest, der allerdings über die Frequenzen Hz reichte. Auf den histologischen Schni

das Cortische Organ ollständig, auch die Macula sacculi und die Stria vascularis wiesen grosse Missbildungen auf. Der Utriculus und die Bogengänge hatten hingegen eine normale Struktur

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MITTELOHRSCHLEIMHAUTVERÄNDERUNGEN BEI EXPERIMENTELLER DYSFUNKTION DER SCHILDDRÜSE

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Es werden die Veränderungen der Mittelohrschleimhaut der Ratten bei experimenteller Dysfunktion der Schilddrüse beschrieben. Durch die Fütterung der Tiere mit Propylthiouracil (PTU) beziehungsweise Thyral wurde eine Hypo- oder Hyperthyreose provoziert. Diese Veränderungen, sowohl in ihrer Reihenfolge als auch in ihrer Art, entsprechen den üblichen Veränderungen der Respirationsschleimhaut und sind für die Dysfunktion der Schilddrüse nicht charakteristisch. Sie beweisen: 1. dass die Mittelohrschleimhaut nur eine reduzierte Respirationsschleimhaut darstellt, und 2. dass die Reaktionen dieser Schleimhaut in erster Linie von ihrem eigenen morphologischen Aufbau und nicht von der Art der Noxe abhängig sind.

Die Dysfunktion der Schilddrüse führt bekanntlich nicht nur zu gewissen Veränderungen der Respirationsschleimhaut (Bryant, Gušić, Laskiewicz, Proetz, Walsh etc.) sondern auch zu Hörschädigungen. Laskiewicz (1951) hat bei solchen Fällen schon vor Jahren auf die gleichzeitigen Veränderungen der Mittelohrschleimhaut aufmerksam gemacht und diese mit der gestörten Hörfunktion in Zusammenhang zu bringen versucht. Im Anschluss an unsere schon seit Jahren durchgeführten Untersuchungen der Pathophysiologie der Respirationsschleimhaut, haben wir an ihr auch die Veränderungen bei der Dysfunktion der Schilddrüse beim Menschen wie auch im Experiment untersucht. Wir haben in diese Untersuchungen natürlich die Mittelohrschleimhaut einbezogen, da sie ja nur einen teilweise reduzierten Teil der Respirationsschleimhaut darstellt. Über die experimentellen Untersuchungen dieser Art möchten wir hier kurz berichten.

Für das Experiment wurden 75 männliche weisse Ratten vom VM Zuchtstamm (Abkömmlinge der Wistarratten) verwendet. Zu Beginn des Versuches waren die Tiere etwa 3 Monate alt. Ihr Körpergewicht schwankte zwischen 220 und 310 g. Der auriculo-palpebrale Reflex war bei allen vorhanden. Nach der Methode der zufälligen Auswahl wurden sie in 3 Gruppen eingeteilt:

I Gruppe: Bei 25 Ratten wurden auf 200 g Standardlaboratoriumsnahrung 1,6 g getrocknete Schilddrüsensubstanz (Thyral" Prolek, Beograd) verabreicht. Die individuelle Tagesdosis lag bei 0,2 g getrockneter Schilddrüsensubstanz. Dem Standardfutter wurde kein zusätzlicher Vitamingehalt beigefügt.

II Gruppe: Bei 25 Ratten wurde auf 100 g Standardfutter 0,2 g Propylthyouracil (PTU Propocyl, Rhenania Kali-Chemie Hannover) verabreicht. Die individuelle Tagesdosis lag bei 25 mg.

III Gruppe: 25 Ratten wurden mit der Standardlaboratoriumsnahrung ohne irgendwelche zusätzlichen Beigaben, gefüttert. Sie wurden als Kontrollgruppe verwendet.

Alle Tiere lebten unter den gleichen Bedingungen. Wasser bekamen sie in beliebiger Menge. Je zwei Tiere von jeder Gruppe wurden am Ende der 1., 2., 3 4., 6. und 8

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Abb 1 Normale Mittelohrschleimhaut der Ratte. — Es ist eine reduzierte Respirationsschleimhaut mit typischen zylindrischen Flimmer und Becherzellen, intraepithelialen *Drüsen* und einer durch lockeres Bindegewebe gebildeten Submucosa. Die Basalmembran ist auch teilweise sichtbar

nach dem Beginn des Expermentes geopfert.

Das Körpergewicht wurde regelmässig kontrolliert und der Sauerstoffverbrauch nach der Methode McLegan und Sheahan in der Modifikation nach Tomich und Woollett am Anfang jeder Woche und nachher alle 14 Tage nachuntersucht.

Bei den hypothyreotischen Tieren wurde eine bemerkbare Abnahme des Sauerstoffverbrauches vom Ende der zweiten Woche an festgestellt. Auch das Körpergewicht zeigte eine signifikante Herabsetzung. Dagegen konnte in der Gruppe der hyperthyreotischen Versuchstiere schon am Ende der ersten Woche eine

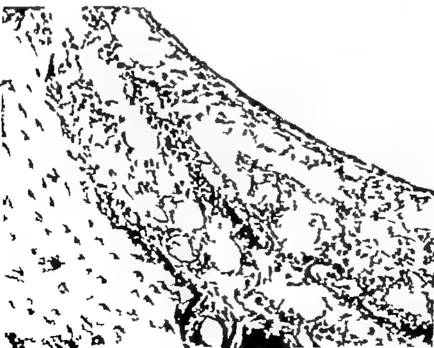


Abb 2 Mittelohrschleimhaut der Ratte bei Hypothyreose — 4 Woche. Vergrösserung 180. *Ödem* und *verbreiterte* Lymphbahnen und angedeutete Kleinzellerinfiltration in der Submucosa. Einreihiges Plattenepithel.



Abb 3 Mittelohrschleimhaut der Ratte bei Hypothyreose. — 19 Woche. Vergrosserung 180. Inflammationsgranulom der Schleimhaut mit noch teilweise erhaltenen Respirationsepithel.

starke Vergrösserung des Sauerstoffverbrauches mit gleichzeitig gleichmässiger Gewichtsabnahme bemerkt werden, was bis zum Ende des Experimentes andauerte Die Tatsache, dass keines der Tiere die 8 Woche überlebte, zeigt die Grösse der Hyperthyreose.

Die Tiere wurden durch Verblutung in Nembutal Anästhesie getötet, der Kopf sofort abgeschnitten Nach der durchgeführten Fixation in 5% Formalinlösung wurde der Knochen in 10% Trichloressigsäure dekalziniert und in Paraffin eingebettet. Die 12μ dicken histologischen Serienschnitte wurden mit Haemalaun Eosin und nach der Mallory Methode gefärbt.

Wenn wir uns jetzt den histologischen Befunden selbst zuwenden wollen, so müssen wir



Abb 4 Mittelohrschleimhaut der Ratte bei Hyperthyreose. — 8 Woche. Vergrosserung 54 Schleimhautverdickung als Folge einer massiven Kleinzelleninfiltration mit teilweise defektem Plattenepithel, verbreiterten Lymphbahnen und neugebildeten Kapillaren der ödematosen Submucosa. Der Mittelohrraum ist mit Transsudat ausgefüllt.

vorerst wieder feststellen, dass sie sich in den Grenzen der Reaktionsweise der Respirationsschleimhaut bewegen, wie wir das in einer Reihe unserer früheren Arbeiten an der Schleimhaut der Atmungswege feststellen konnten. Dies spricht noch mehr für die Richtigkeit der Annahme, dass wir es bei der Mittelohrschleimhaut eigentlich mit einer reduzierten Respirationsschleimhaut zu tun haben.

Unter normalen Bedingungen enthält die Mittelohrschleimhaut der Ratte alle charakteristischen Elemente einer Respirationsschleimhaut, nur eben in reduzierter Menge Es sind dies. die zylindrischen Epithelzellen mit Zilienbesatz, sezernierende Becherzellen an einzelnen Stellen namentlich in der Nähe der Tubenöffnung, manchmal auch eine basale Schicht, durch eine angedeutete Membran nach unten abgegrenzt und zuletzt eine unterschiedlich dicke Submucosa mit Drüsen Lymph- und Blutgefässen. Eine solche Schleimhaut wird, je weiter wir uns von der Tubenöffnung entfernen, immer dünner um an den entlegendsten Punkten den Charakter eines Mukoperiostes anzunehmen Entlang des Limbus bleibt ihre Dicke meistens erhalten.

Bei der Hypothyreose zeigen sich die ersten Anzeichen einer Reaktion erst während der [illegible] Woche nach Anfang des Experimentes, bis sie, durchschnittlich am Ende der 16 Woche, ihre [illegible] Ausbildung erhalten Das zylindrische Zinepithel verliert an Höhe, es wird kubisch, um zuletzt in ein einschichtiges Plattenepithel überzugehen. Gleichzeitig wird auch der Zilienbesatz immer spärlicher um zuletzt völlig zu verschwinden. Es entwickelt sich gleichzeitig ein Ödem der Submucosa, zu dem später eine Neubildung von Kapillaren und Lymphbahnen hinzu kommt. Zuletzt entwickeln sich Zeichen einer lokalisierten Entzündung, die oft am Limbus und in der Umgebung der Tubenmündung lokalisiert ist. Bei weiterer Entwicklung kommt es zur Bildung richtiger Entzündungsgranulome mit völliger Zerstörung der oberflächlichen Schleimhaut.

Bei der Hyperthyreose melden sich die Veränderungen viel früher und zwar schon in der 3 Woche nach Anfang des Experimentes. Dem verstärkten Metabolismus entsprechend sind auch die Schleimhautveränderungen viel intensiver und ihrer Verbreitung nach viel umfangreicher Die kleinzellige Infiltration tritt schon viel früher auf und das Bild der Entzündung beherrscht das Feld. Das hohe Respirationsepithel ändert sich sehr schnell in ein ganz niedriges Plattenepithel, die Submucosa wird durch die Inflammationszellen verdickt. Es bilden sich in dem neugebildeten Granulationsgewebe neue Kapillaren und die bestehenden Lymphgefässe werden stark verbreitert. Das Mittelohrlumen wird durch entzündliches Transsudat ausgefüllt. Für die Schwere des Insultes spricht auch die Tatsache, dass kein Tier die [illegible] Woche des Experimentes überlebte.

Wenn wir jetzt einen Vergleich mit den Veränderungen an der menschlichen Mittelohrschleimhaut bei den Dysfunktionen der Schilddrüse wagen wollen, obschon Schnelligkeit und Umfang der Reaktion bei weitem nicht die analogen Ausmasse erreichen muss, so werden uns doch die Ohrsymptome unserer Kranken, wie das Ohrensausen, der Druck oder das Gefühl einer Verstopfung, etwas verständlicher erscheinen. Es wird uns auch klarer warum bei der Hyperthyreose diese Erscheinungen stärker auftreten, aber sich auch meist schneller verlieren, wogegen sie bei der Unterfunktion der Schilddrüse langsamer in Erscheinung treten, aber sich dann meist auch nicht mehr wesentlich zurückbilden.

Für unsere Betrachtungen hier scheint [illegible] uns aber wichtig, noch einmal hervorzuheben dass sich einerseits die Reaktionsweise der Mittelohrschleimhaut auch bei diesen endogenen Noxen in demselben Rahmen vollzieht und sie sich andererseits auf analoge Weise wie wir es an der übrigen Respirationsschleimhaut beobachten können, entwickelt.

SUMMARY

By the administration of propylthiouracil (PTU) or thyral [illegible] rat, hypo- or hyperfunction of the thyroid gland were provoked. The changes in the mucous membrane of the middle ear are described The se-

[illegible] and characteristics of these features do not result from common changes in the respiratory mucous membrane of these animals; hence they are not specific for the dysfunction of the thyroid gland. In addition, it was established that (1.) the mucous membrane of the middle ear is, judged by its characteristics, [illegible] reduced respiratory mucous membrane, and (2.) that the reaction of the respiratory mucous membrane depends primarily on its morphological structure and not on the type of noxa to which it is subjected.

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ALKALINE PHOSPHATASE ACTIVITY IN THE EFFERENT NERVOUS SYSTEM OF THE INNER EAR

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Using Burstone's (1958) alkaline phosphatase staining method, the efferent nervous system in the inner ear of guinea pigs was histochemically demonstrated in surface preparation and sectioned specimens. The findings were essentially similar to those of efferent innervation described by previous authors who used an acetylcholinesterase staining technique. The significance of alkaline phosphatase in the nervous system is briefly discussed.

Since Churchill *et al.* (1956) histochemically demonstrated a high concentration of acetylcholinesterase activity in the efferent nervous system of the organ of Corti, this characteristic has been used to trace the course of nerve fibers or to localize the nerve endings in vari- … species (Schuknecht *et al* 1959 Hilding Versäll, 1962 Smith & Rasmussen, 1963 [illegible] *et al.* 1965 Nomura & Schuknecht, 1965 Ishii *et al* 1967 Ishii & Balogh, 1968) The efferent nerve fibers and endings can also be visualized with Maillet's zinc iodide technique (1962) However the function and morphology of the efferent nervous system in the inner ear are not precisely known. The present study intends to demonstrate the apparent selective activity of alkaline phosphatase in the efferent nervous system of the inner ear of the guinea pig, using Burstone's (1958) simultaneous coupling azo dye method and briefly to discuss the role of alkaline phosphatase in the nervous system.

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MATERIAL & METHOD

Twenty albino guinea pigs weighing approximately 250 g were used in this study After decapitation under light ether anesthesia, the inner ears were immediately removed in toto and fixed in 4 % neutral formol-calcium or cold 80 % alcohol solution for 24 hours at 4 C. For frozen section specimens the tissues were decalcified in a 5 % buffered solution of EDTA for 4 to 7 days at 4 C (Freiman, 1954) When decalcification was completed, the tissues were washed thoroughly in cold physiological saline solution for 10 minutes. The blocks of decalcified inner ears were frozen on dry ice and mounted for sectioning in a cryostat (−30 C) with a rotary microtome at 10 μ The midmodiolar sections were put on clean cover glasses, thawed slowly and dried at room temperature for 2 or 3 minutes. The sections were then placed in a 1 % solution of magnesium chloride for 2 to 4 hours for reactivation of the enzyme (Freiman, 1954). The sections were incubated for 2 to 3 hours at 4 C in media containing Naphthol AS-TR phosphate as a substrate and Fast Red Violet LB salt as a coupler (Burstone, 1958) In most cases, methyl green was used as a nuclear stain.

For surface preparations the soft tissues were removed from the cochlea and vestibular apparatus under a dissection microscope, and the tissues were incubated for 1 to 2 hours in



Fig. 1 Frozen section of the organ of Corti (basal turn). Sites of alkaline phosphatase activity are indicated by azo dye deposits. Intense enzyme activity is seen in the nerve endings (*NE*) at the base of the outer and inner hair cells, inner (*ISB*) and outer (*OSB*) spiral bundles and radially transversing tunnel nerve fibers (*TRF*) towards the outer hair cells. A considerable number of azo dye deposits are also distributed in the tympanic lamella. All cells of the organ of Corti show no histochemical reaction. Nuclei are stained with methyl green. 790.

the same media as that of the sectioned specimens. For control specimens, Barka's (1963) methods were used to distinguish false positive staining from genuine enzyme reactions. The specimens were incubated in a substrate-deficient medium, or treated with 5% trichloroacetic acid or 15% acetic acid or diluted nitric acid for 2 to 5 minutes. The specimens of sur



Fig. 2 Surface preparation of the organ of Corti (basal turn). Outer (*OSB*), inner (*ISB*) and tunnel (*TSB*) spiral bundles and radially transversing tunnel nerve fibers (*TRF*) of the organ of Corti are demonstrated. Nerve endings (*NE*) of outer cells are visualized as three rows. *VS* vas spirale; *C* capillary 360.



Fig 3 Apical turn. The first row of outer hair cells shows strong reaction in the nerve endings (*NE*), whereas the second and third rows show none. *ISB*, inner spiral bundle; *VS* vas spirale. 490.

face preparations and sections were mounted in glycerine jelly or PVP medium for light microscopic examination.

FINDINGS

Granular or amorphous brilliant red dye deposits were seen at the sites of alkaline phosphatase activity in the surface preparations and frozen section specimens. Control specimens showed no histochemical reaction.

In the lower turns of the organ of Corti, remarkable alkaline phosphatase activity was ...erved in the nerve endings at the base of inner hair cells, and also was strongly detectable in the nerve fibers of the inner outer and tunnel spiral bundles (Figs. 1 and 2). However throughout the cochlea, the localization of this enzyme activity was not easily distinguished between the nerve endings of inner hair cells and nerve fibers of inner spiral bundles. Among the outer hair cells, the nerve endings of the first row were the most dominant in size and enzyme activity

In the upper turns of the organ of Corti, alkaline phosphatase positive innervation of the outer hair cells gradually decreased until no enzyme positive innervation was present in the

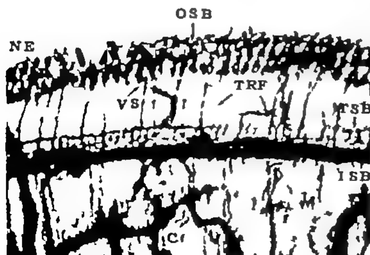


Fig 4 Apical turn. The inner (*ISB*), outer (*OSB*) and tunnel (*TSB*) spiral bundles and tunnel radial nerve fibers (*TRF*) are clearly demonstrated. Only nerve endings (*NE*) of the first row of outer hair cells are visualized. *VS* vas spirale; *C* capillaries. 360



Fig. 5 Osseous spiral lamina (upper basal turn). Intense activity of alkaline phosphatase is seen in the intraganglionic spiral bundle (*IGB*) and among the spiral ganglion cells (*SGC*). *OC* organ of Corti. *C* capillary ×110.

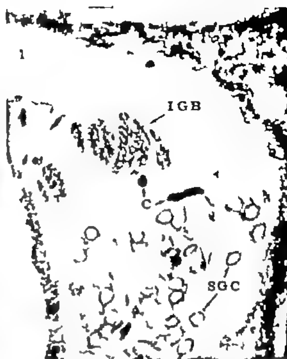


Fig. 6 Rosenthal's canal. Enzyme activity is restricted to intraganglionic spiral bundles (*IGB*) and peripheral areas of spiral ganglion cells (*SGC*). All other nerve fibers fail to show alkaline phosphatase activity. *C* capillaries. ×270.

apical end. These reaction deposits were much less in the outer row in comparison to the inner row of outer hair cells (Figs. 3 and 4). These findings were essentially similar to those of efferent innervation described by Ishii & Balogh (1968).

Strong activity of alkaline phosphatase was also observed in the intraganglionic spiral

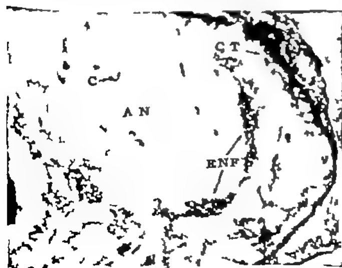


Fig. [illegible] Modiolus. Intense alkaline phosphatase activity is mainly located in the efferent nerve fibers (*ENF*) and the capillaries (*C*) in the acoustic nerve and the connective tissue (*CT*). No histochemical reaction is present in the afferent nerve fibers. *AN* acou[illegible] ×135

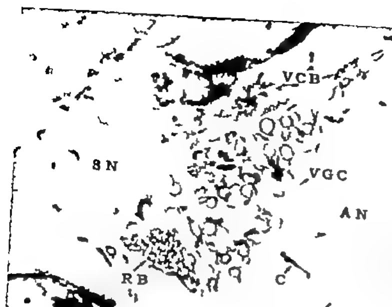


Fig 8 Frozen section of stato-acoustic nerve. Rasmussen's *bundle* (*RB*) and vestibulo-cochlear bundle (*VCB*) showed strong alkaline phosphatase activity A peripheral localization of this enzyme on or near the cell membrane is seen in the vestibular ganglion cells (*VGC*). *C* capillary *AN* acoustic nerve *SN* saccular nerve. × 150.

bundles and in the peripheral areas of the spiral ganglion cells of Rosenthal's canals (Figs. 5 and 6) The walls of the vas spirale and capillaries below the inner pillar showed remarkable enzyme activity

In the modiolus, intense alkaline phosphatase activity was restricted to the efferent nerve fibers, the capillaries in the acoustic nerve and the fibrous connective tissues surrounding the acoustic nerve trunk (Fig. 7)

In the vestibular apparatus, azo dye granules were sparsely distributed throughout the cytoplasm of sensory epithelial cells, but they seemed to be concentrated at the base or immediately surrounding these sensory cells, probably in the nerve chalices around the Type I sensory cells of the crista ampullaris and the maculae utriculi and sacculi. The hairs of sensory cells and the capillaries underneath the sensory epithelium also showed remarkable activity of alkaline phosphatase (Fig. 8)

There were relatively few nerve fibers containing alkaline phosphatase in the vestibular labyrinth.



Fig 9 Facial nerve. Nerve fibers containing alkaline phosphatase (*arrows*) scattered at random throughout the facial nerve. *C* capillary *CT* connective tissue. 180



Fig. 10 Sensory epithelium of the macula utriculi. Azo dye granules are sparsely distributed throughout the cytoplasm of sensory cells, but they seem to be concentrated at the base or immediately surrounding these sensory epithelial cells (*arrows*) and stereocilia (*S*). It is interesting to note that the enzyme positive nerve fibers underneath the sensory epithelium are few in number. *C* capillaries. 400.

Considerable numbers of alkaline phosphatase positive nerve fibers were scattered at random throughout the facial nerve (Fig. 9)

This enzyme activity has been consistently observed on or near the cell membrane in the vestibular and geniculate ganglion cells (Fig 10)

DISCUSSION

It has been widely believed that acetylcholinesterase activity seems to be characteristic for the efferent nervous system of the inner ear because high acetylcholinesterase activity can be selectively demonstrated in the efferent nervous system of various organs. But it seems from the present study that the efferent nervous system in the inner ear of the guinea pig is demonstrable with the staining method of not only acetylcholinesterase but also alkaline phosphatase

For demonstration of the efferent nerve endings and fibers in the inner ear of guinea pigs, Engström *et al.* (1966) employed the zinc iodide-osmium technique of Maillet (1962) In an electron microscopic study Akert & Sandri (1968) confirmed that this staining method selectively depicts the synaptic vesicles in the subfornical organ and neuromuscular junction. They discussed a possible relationship between this zinc iodide-osmium vesicular staining and cholinergic transmission. However some recent electrophysiological studies deny that the efferent nerve fibers are cholinergic (Desmedt & La Grutta, 1963 Katsuki *et al* 1965 Tanaka & Katsuki, 1966)

The biological significance of alkaline phosphatase has not been as well understood as that of acetylcholinesterase. The complex regional and cellular distribution patterns revealed by histochemical methods make it even more difficult to interpret the distribution of this enzyme in terms of known physiological functions.

In the inner ear of the guinea pig, most

line phosphatase activity was demonstrated in the capillaries and arterioles with surface pre parations (Nomura & Hiraide 1968) and frozen sections. It is possible that alkaline phosphatase in the blood vessel might be re lated to "active transport" as in the proximal tubules of the kidney and in the mucosa of the small intestine (Romanul & Bannister 1962)

According to Winkelman's observations (1960, 1962) alkaline phosphatase and cholinesterase reactions were always present in the sensory end-organs of mammalian skins. Alkaline phosphatase has also been ascribed a de finite role in the conduction of nervous impulses, for it was demonstrated in the region of the synapses (Bourne, 1958 Tewari & Bourne, 1963) and in nerve endings, incapsulated cor puscles and motor endplates (Portugalov 1955) In Bourne's opinion (1958) the pre sence of alkaline phosphatase in the synapses is evidence that fresh energy is constantly being supplied here and the possible role of this enzyme is synaptic transmission. But it is inter esting to note in the present study that alkaline phosphatase activity was seen not only in the nerve endings, but also in all other efferent nerve fibers of the inner ear

The alkaline phosphatase activity of afferent coustic and vestibular nerve fibers was almost gative or very weak, except in the peripheral eas of spiral and vestibular ganglion cells where strong activity was detectable. However it is clear that an obvious difference in alka line phosphatase activity exists between the ef ferent and afferent nervous systems in the in ner ear

The universal appearance of alkaline phos phatase activity in the efferent nervous system of the inner ear of guinea pigs suggests some distinct function of this enzyme. This method could possibly be used to demonstrate the re lationship between the atrophy of sensory cells and the degeneration of blood vessels It is necessary to accumulate more information about the direct relationship between the func tion of the efferent nervous system and the enzymes demonstrated by histochemistry

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ZUSAMMENFASSUNG

Das efferente Nervensystem im inneren Ohr von *Meerschweinchen* wurde in *Oberflächenpräparaten* und Gewebeschnitten mithilfe der Burstone'schen Alkaliphosphatase Färbetechnik (1958) dargestellt. Die Ergebnisse stimmten im wesentlichen mit denen über die efferente Innervation überein, die bereits von anderen Autoren, die eine Acetylcholinesterase Färbetechnik verwendeten, beschrieben worden sind. Die *Bedeutung von Alkaliphosphatase* im Nervensystem wurde kurz diskutiert.

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FUNCTIONING REMOBILIZATION OF VOCAL CORDS IN CATS WITH PERMANENT RECURRENT LARYNGEAL NERVE PARESIS

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From a strictly neuro-physiological standpoint it is shown why a functioning re-innervation of laryngeal muscles is not to be expected after degeneration of the recurrent laryngeal nerve (r.l.n.). It is also shown why at such degeneration the r.l.n., paradoxically as it may seem, should definitely not be used in attempts to re-innervate the larynx. For the same reasons the chances of getting the desired function using an anastomosis with some other nerve and the r.l.n. as the distal part are exceedingly small and accidental.

The facts that the posticus muscle has a respiratory function, i.e. contracts at inspiration and that the characteristics of a muscle are mainly determined by its nerve and not *vice versa* have been the prime reasons for implanting into the posticus muscle a e originally belonging to a muscle with the same ratory function, the phrenic nerve, which is at expiration. In this way ocal cords of cats, permanently deprived of their r.l.n. were made to bduct at inspiration, adduction at expiration being performed by the crico-thyreoid muscle. The method is recommended in the case of patients with bilateral paresis of the r.l.n. in order to reduce the breathing difficulties without obtaining an accompanying deterioration of the voice

Usually patients with bilateral paresis of the recurrent laryngeal nerve (r.l.n.) have considerable difficulties. If the vocal cords are in a median position on both sides the patient cannot breathe and tracheostomy is of vital importance. If one of the cords is in the median and the other cord in the paramedian position the patient has a fairly good voice but breathing is considerably impaired. If both cords are in the paramedian position the patient can breathe freely at rest and the voice may be adequate but not seldom with a more or less strong tendency towards breathiness.

The greatest troubles encountered in patients with bilateral r.l.n paresis are respiratory ones. If such patients lead a quiet and physically inactive life they sometimes get along well, but if they wish to live more normally and be able to put up with moderate physical strain, this is not possible. The voice problems in such patients are moderate and can usually be eliminated by phoniatric treatment. For the respiratory difficulties several methods, most of them including arytenoidectomy have been used (King, 1939 Kelly 1941 Woodman, 1946 Thornell, 1948) In common with most of these methods is the fact that the resulting permanent widening of the glottis, even if it increases the breathing capacity is accompanied by a deterioration of the voice. Since improvements of these methods are suggested almost every year the clinical results are evidently not satisfactory and a search for new methods precluding a fixation of the cords is warranted.

Several attempts have been made to reinnervate the paretic muscles. Principally two ways

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to achieve this have been tried, (*a*) repair of the function of r.l.n. and (*b*) the making of an anastomosis between a foreign nerve and the r.l.n.

(*a*) Horsley (1909) described complete return of cord function following neurorrhaphy made three months after a trauma to the nerve. Since then a small number of successful reports on neurorrhaphy have been published (Lahey 1928), but in spite of this the method has never been generally accepted. Very recently new experiments in this field have been made (Doyle *et al.*, 1967, Miehlke *et al.* 1967, Gordon & McCabe, 1968, Mounier-Kuhn *et al.* 1968). All these studies were done on dogs and in most instances the results were checked by electrical stimulation and/or the observation of active movements of the cords. From none of these reports, however, was it obvious that the return of cord movements was of functional value.

(*b*) Reports on various nerve anastomoses where a foreign nerve was connected to the distal part of the r.l.n. are fairly common (Serafini & Uffreduzzi, 1914, Frazier 1924, Colledge & Ballance, 1927, Lahey & Hoover 1938, Doyle *et al.* 1967, Miehlke *et al.* 1967, Berendes & Miehlke, 1968) but with the same dubious functional success as in neurorrhaphy.

The main reasons for the difficulties in achieving a functional innervation of the muscles previously innervated by r.l.n. are twofold. (*a*) A denervated muscle is reinnervated by any motor nerve with no preference to its own (Weiss & Hoag, 1946, Bernstein & Guth, 1961). (*b*) The r.l.n. innervates four different muscles one of which, the posterior cricoarytenoid muscle, is antagonistic to the others, i.e. it relaxes when the other three muscles contract and *vice versa*. For these reasons the nerve axons following regeneration or a nerve anastomosis will at random reinnervate muscle fibres belonging to abducting and adducting muscles (Siribodhi *et al.* 1963, Hiroto *et al.*, 1968).

Which vocal cord motions are to be desired? According to Berendes & Miehlke (1968) the human larynx has at least nine functions: respiratory, phonatory, circulatory, protective, fixative, deglutitory, tussive, expectorative and emotional. Of these functions two are commonly regarded as the most important ones, the respiratory function, which is vital, and the phonatory function. Respiration goes with abduction of the vocal cords and phonation with adduction. As already mentioned phonation is not a main problem in patients with bilateral r.l.n. paresis simply because the vocal cords are more or less adducted by the action of the crico-thyroid muscle. It is therefore only of secondary importance to increase the adducting ability of one or both of the vocal cords. A far more essential problem is how to produce abduction of at least one vocal cord without interfering with the already existing adduction. The only way to achieve this is by reinnervation, but for the reasons given the reinnervation should be a selective one restricted to the posticus muscle, i.e. the only abducting muscle of the larynx. Such a reinnervation can be performed in two ways.

A. Neurorrhaphy with the laryngeal part consisting of the very branch of the r.l.n. to which belongs the posticus muscle. If the r.l.n. is chosen as the other part it is evidently necessary to find the fascicles of the nerve intended for the posticus muscle and this is experimentally as well as clinically very difficult or impossible to do. Sunderland & Swaney (1952) investigated the intra-neural topography of the r.l.n. in man and found that the nerve funiculi repeatedly changed their positions in the nerve trunk as did the nerve fibres. Nor can it b[illegible] said that the fibres controlling any particula[illegible] functional mechanism occupy a constant secto[illegible] of the nerve. It is therefore necessary [illegible] wishes to use the r.l.n. to make the [illegible] rhaphy where the nerve has branch[illegible] nical difficulty involved in such [illegible] rhaphy is that it is not easy to [illegible] ryngeal branch belonging to the [illegible] cle and to connect the two nerves [illegible] aging them. Thus no reports are [illegible] a neurorrhaphy in which the



Fig 1 View of larynx from cat 6 at *direct laryngoscopy* Paresis of left r.l.n. + implantation of left phrenic nerve into left posticus muscle. Quiet breathing in *1* expiration and *2* the following inspiration *3* hyperventilation, inspiration. *4–6* paresis of both r.l.n., hyperventilation. *4* expiration and *5* the following inspiration. *6* as before + implanted phrenic nerve cut, inspiration but no abduction.

a sufficiently long distance to allow it to reach the back of the larynx without stretching. The final 8 cats, *in which at autopsy the implanted* nerve was found in the posticus muscle, showed mobility of both vocal cords, i.e. abduction at inspiration. The first sign of function on the implanted side was a rather characteristic tremor of the arytenoid region. The tremor which was observed by direct laryngoscopy did not appear until several months after nerve implantation. About two months later the vocal cord of the operated side was seen to abduct at inspiration (Table 1)

Measurements made from the film of the time when the glottis was opened showed that abduction always started later on the operated side while adduction was completed synchronously The duration of abduction on the operated side expressed in percentage of the duration on the non-operated side varied between 50 and 80% in those cats where numerical estimations could be made (Table 1)

The weights of the posticus muscles were fairly similar (Table 1), the weight ratio of the operated muscle to the non-operated one, showed that the atrophy which must have occurred at the initial denervation was eliminated by the implanted nerve.

The control of diaphragmatic movements showed a considerable decrease of the mobility of the *diaphragm on the operated side* The position of the diaphragm was also more cranial than on the non-operated side indicating a partial paresis.

A short description follows of those cats which were filmed.

Cat 5 Direct laryngoscopy immediately before taking the moving picture showed that the vocal cord on the operated side abducted less than that of the contra-lateral side. When the cat inhaled an increasing concentration of CO_2 from the bag on the tracheal cannula increased cord movements were observed, to about equal extent, on both sides Cutting the remaining r.l.n. stopped cord movements at the control side while the side with the nerve implant still moved as before. When the implanted nerve was cut both cords were completely immobilized. At autopsy the implanted phrenic nerve was found in the posticus muscle

Cat 6 Strong movements, of the same magnitude as in the control side were seen in the cord of the operated side at direct laryngoscopy immediately preceding filming and during analysis of the moving pictures. This means that the cord of the operated side abducted at inspiration as much or even somewhat more than the contra-lateral cord (Fig. 1) The findings after severance of the nerves and at autopsy were as described above

Cat 7 Direct laryngoscopy preceding filming showed small movements in both cords of about the same magnitude. Remarkable in this cat were the small movements also of the control cord. Thus it was difficult to see any increase in abduction at induced hyperventilation even if the movements were somewhat larger than on the operated side. This made it impossible to estimate the time of the cord movements with sufficient accuracy Cutting the nerves and the findings at autopsy gave the same results as in the previous cats. Both posticus muscles were of the same weight but only about one half as heavy as in the other cats.

Cat 8 Abduction at inspiration was less pronounced on the operated side and during induced hyperventilation the cord abducted only as much as did the cord on the contra-lateral control side before hyperventilation. After sectioning of the nerves and at autopsy the results were as described for cat 5

DISCUSSION

The aim of the present investigation was to obtain remobilization with abduction at inspiration and adduction at expiration of vocal cords which had been immobilized by cutting the r.l.n. Such a restoration of function was achieved in cats by the implantation of the phrenic nerve of one side into the paretic homo-lateral posticus muscle. Examination of the cats 8–20 months later showed that the vocal cord on the side with the nerve implant abducted to about a similar extent as that of the contra-lateral control cord during inhalation even if the movement of the cord with the implant was somewhat slower than that on the other side. Thus the use of the phrenic nerve as a substitute for the original r.l.n. restored the function of the paretic posticus muscle to almost normal. The results of the present study are therefore superior to those previously reported from experiments in which neurorraphy or implantation of the r.l.n. were made into the posticus muscle, since in those instances vocal cord movements synchronous with respiration were not achieved.

The possibility that the posticus muscle had changed its speed characteristics, metabolism etc. under the influence of the nerve implant cannot be answered until these parameters have been quantitatively determined. However it is noteworthy that in one of the cats

posticus muscle on the implanted side had been abducting continuously during respiration for at least 15 months and thus indicates that the mechanical and metabolic characteristics of the muscle had not changed, at least not to such an extent as to impair function.

In 15 cats out of 23 the implanted phrenic nerve failed to innervate the posticus muscle As previously mentioned this failure presumably resulted from a difficulty to dissect the nerve for a sufficiently long distance to allow it to reach the posticus muscle without stretching. In man the phrenic nerve emerges one segment higher in the neck and it should be possible to move without difficulty the nerve or a nerve fasciculus, to the back of the larynx. However it is obvious that this operation has actually to be tried in man before its useful ness and clinical value can be assessed. It should be stressed that if an implantation is to result in functioning reinnervation it is nec essary to know that the muscle in question is denervated Innervation in an immobilized vocal cord should always be suspected if the cord shows no sign of atrophy as it is known that a nerve can have a trophic effect on the muscle in spite of the fact that no contractions of the muscle can be elicited by the nerve (Fex, 69) Elsberg (1917) and many after him e found that if a muscle is innervated it is possible to make the muscle contract by a new nerve, and it is therefore advisable to ascertain denervation by for instance E.M G or by cutting the r.l.n. for good before implantation is made

ZUSAMMENFASSUNG

Mit Hilfe strikt neurophysiologischer Erwägungen wird begründet, warum nach der Degeneration des N laryngeus recurrens eine funktionierende (spontane) Reinnervation der Kehlkopfmuskeln nicht zu erwarten ist. Es wird auch erwiesen, warum bei einer solchen Degeneration anscheinend paradoxer Weise gerade der N recurrens zu Reinnervationsversuchen vollkommen ungeeignet ist. Aus denselben Gründen erscheinen die *Aussichten auf erwünschte Funktion* nach dem Anlegen einer Anastomose zwischen einem anderen Nerven und dem distalen Ende des N recurrens als ausserordentlich gering und zufällig.

Der M crico-arytaenoideus posterior (Posticus) nimmt an der Atmung teil, d. h. er wird beim Einatmen kontrahiert. Seine Eigenschaften werden — je die eines jeglichen Muskels — hauptsächlich durch seinen Nerven bestimmt und nicht umgekehrt. Dies sind die vorwiegendsten Gründe, in den Posticus einen Nerven einzupflanzen, der ursprünglich zu einem Muskel mit der gleichen Atmungsfunktion gehört, nämlich den N phrenicus, der bei der Atmung nicht aktiviert wird. Auf diese Weise konnten die Stimmbänder bei Katzen, die durch ständige Denervation ihrer Recurrensnerven beraubt waren, beim Einatmen wieder zum Abduzieren gebracht werden, während die Adduktion beim Ausatmen durch den M crico-thyreoideus erfolgte. Eine Verwendung dieser Methode wird bei Patienten mit doppelseitiger Lähmung des N recurrens empfohlen, um ohne gleichzeitige Verschlechterung der Stimme die Atmung zu erleichtern.

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THE CORRELATION BETWEEN THE RADIOLOGICAL EXAMINATION AND THE IRRIGATION FINDINGS IN MAXILLARY SINUSITIS

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The ENT-doctor has a right to demand a high degree of confidence from the radiological examination in establishing the diagnosis of maxillary sinusitis, with or without fluid content. Previous investigations have compared the radiological changes with the irrigation findings. The present investigation reports a similar comparison but correlates further the fluid demonstrated by radiological examination with secretion demonstrated by irrigation. Fluid content was demonstrated by irrigation in 86% of the completely opaque sinuses. In cases with radiological mucous membrane thickening, secretion was demonstrated by irrigation i 60% but among these cases a fluid level was only demonstrated radiologically in 24% when four standard projections are used. If a 5th projection is added, i.e. occipitomental with the patient recumbent and the affected side downwards, the radiological confidence of demonstrated fluid increases to 88% This projection is recommended for routine purposes in cases where four standard projections disclose mucous membrane thickening but no fluid.

Radiological examination is generally used to establish the diagnosis of sinusitis either by plain radiograms by tomography or by contrast radiography However radiological examinations are time-consuming for the patient including consultation in another department, sometimes in another hospital. Consequently if the radiological examination is to defend its place in diagnosis, it is justified to demand that it can be made quickly and with a high degree of diagnostic accuracy

There are relatively few investigations published on the correlation between radiological findings and the lavage content of subsequent irrigations of maxillary sinusitis. Ballantyne (1946) examined 66 cases with positive findings recorded on X-ray examination Fifty-eight (88%) of these showed evidence of sinus pathology on puncture and lavage" The radiological technique is not described, nor the interval between the radiological examination and the puncture of the maxillary sinus.

In 1949 Ballantyne & Rowe reported on the correlation between radiological findings and proof puncture in 206 consecutive and unselected cases of chronic maxillary sinusitis. Two radiological projections were used the occipito-mental and the occipito-frontal The material was divided into three main groups: (1) Ten cases with negative radiograms, (2) Eighty eight cases with radiologically opaque maxillary sinuses. (3) Eighty-two cases with radiological appearance of a thickened mucous membrane. The irrigations findings of group I were completely negative, while more than 80 of group 2 and less than 10% of group 3 demonstrated positive findings on lavage

Buch (1949) examined 150 patients with 192 blurred" maxillary sinuses by means of puncture. Sinusitis with secretion was demonstrated in 65% Hinde (1950) reported on the findings in 100 cases of chronic maxillary sinusitis. The radiological examination was made in projections unable to demonstrate fluid levels and the maxillary sinuses were divided into two groups those with uniformly opaque sinuses and those with a thickened mucosa. Secretions were found on proof puncture

and lavage in 80% of the former group and in 16% of the latter. Immediate puncture was made in only 21% of the cases with positive radiograms.

Vuornen *et al.* (1962) in a retrospective study compared the radiological findings with the results of irrigation of 272 maxillary sinuses in 190 patients. Three radiological projections were used with the patient sitting: occipito-mental, occipito-frontal and lateral. Secretion was demonstrated at irrigation in 86% of the cases with completely or intensely opaque maxillary sinuses, in 54% of the cases with mucosal thickening and in 6% of the cases with normal radiological findings. The time interval between the radiological examination and the irrigation is not given.

McNeill (1963) compared the radiological findings with the lavage content from the maxillary sinuses of 150 patients with clinical or radiological evidence of infection. Three projections were used: the occipito-mental, the occipito-frontal and the lateral. In patients with normal radiograms but clinical evidence of infection, secretion was found in 20% at irrigation. In cases with radiological mucosal thickening secretion was demonstrated in 63%. In cases with radiological opaque maxillary sinuses secretion was found in 83%. The interval between the radiological examination and the irrigation was not noted.

The aim of the present investigation was to establish the confidence of standard radiological measures in maxillary sinusitis and if possible to improve the diagnostic measures by further radiological projections. This was done by comparing the radiological findings with immediate irrigation of the affected maxillary sinuses. Previous authors have compared radiological changes with the irrigation finding but have not investigated the radiological confidence of demonstrated secretion which was done in the present investigation.

MATERIAL AND METHOD

The material contains 197 patients, 113 female and 84 male, with 301 maxillary sinuses demonstrating radiological changes, of which 152 on the right and 149 on the left side. The mean age was 31 years (range 3–74 years). All patients attending the ENT-department with a history of or with a clinical picture indicating sinusitis were directly referred to the radiological department within the hospital. The following radiological standard projections were used on the Lysholm skull table: (1) Occipito-mental. (2) Occipito-frontal. (3) Lateral. (4) Full axial. The exposures were made with the patient sitting and horizontal direction of the central beam to demonstrate fluid levels.

The material contains 64 maxillary sinuses examined by an additional 5th projection. This was occipito-mental with the patients' head in a recumbent position and with the affected side downwards. The 5th projection was introduced during the investigation and adopted in sinuses demonstrating mucous membrane thickening but no fluid level on the four standard radiograms. This material is analysed separately. For further technical radiological information the reader is referred to Chidekel *et al.* 1969.

All patients demonstrating any radiological changes in the maxillary sinuses were immediately irrigated through the inferior meatus and the lavage content noted for subsequent comparison with the radiological findings. In cases where the radiological examination did not disclose fluid in any of the five projections, but secretion was demonstrated at irrigation, the amount of secretion was noted. The complete otological and radiological examinations were usually performed in 30 min.

RESULTS

The results of the radiological examination and of the irrigation are given in Table 1. Liquid content was demonstrated at irrigation in 86% of the completely opaque sinuses. In the 8 cases with cysts or polyps on the radiograms, there was no evidence of secretion at irrigation. In the remaining cases with mucous membrane thickening, secretion was demonstrated at irrigation in 60%.

The occurrence of secretion at irrigation was

Table 1 *Radiological and irrigation findings in maxillary sinusitis*

	Radiological examination using 4 standard projections Mucous membrane thickening on radiograms					Radiological examination including the 5th projection Mucous membrane thickening on radiograms		
	1–6 mm	7 mm–<completely opaque	Completely opaque	Polyps	Total	*1–6 mm*	*7 mm–completely opaque*	*Total*
Number of maxillary sinuses	108	102	83	8	301	30	34	64
Secretion observed on radiograms	10 (9 %)	21 (21 %)	0	0	31 (15 %)	16 (53 %)	20 (59 %)	36 (56 %)
Secretion obtained at irrigation	*59 (55 %)*	*69 (67 %)*	*71 (86 %)*	0	*199 (66 %)*	*19 (63 %)*	*24 (71 %)*	*43 (67 %)*
Radiograms correct concerning secretion	*17 %*	*30*		*100*		*84*	[illegible]	*84*

Table 2. *Occurrence of secretion on standard radiograms and irrigation related to different degrees of mucous membrane thickening*

	Mucous membrane thickening on standard radiograms					
	1–3 mm	4–6 mm	7–9 mm	10–12 mm	12 mm—completely opaque	Completely opaque
mber of maxillary sinuses	32	76	43	32	27	[illegible]
ecretion at radiograms	6	11	14	34	15	[illegible]
etion at irrigation	41	61	65	66	74	86

further investigated in relation to the degree of mucous membrane thickening in the diseased maxillary sinuses. The result is noted in Table 2. The occurrence of secretion at irrigation increases with the degree of mucous membrane thickening. The radiological examination, however, can only demonstrate liquid content in relatively few cases, using the four standard projections. Secretion was demonstrated most frequently in the radiograms when there was a mucous membrane thickening of 10–12 mm.

In cases with mucous membrane thickening, the radiological demonstration of fluid increases considerably when the 5th projection is added as seen by Table 1 and Figs. 1 and 2. In cases with a minor degree of mucous membrane thickening, secretion was demonstrated radiologically in 53 % and on irrigation in 63 % and in cases with pronounced mucous membrane thickening secretion was demonstrated radiologically in 59 % and on irrigation in 71 %. The volume of secretion which could not be disclosed by radiological examination in these five projections did not exceed 5 ml.

An analysis was also made to determine the frequency of fluid levels on the radiograms in cases with secretion at irrigation. In no case was fluid demonstrated radiologically which could not be confirmed at irrigation. In cases with completely opaque sinuses, it is obviously



Fig. 1. Fluid in maxillary sinusitis. In (full axial), *b* (lateral) and (occipito-mental) projections mucous membrane thickening with oblique surfaces are demonstrated but no fluid. The occipito-frontal projection, only demonstrating the frontal sinuses, is omitted. In *d* the occipito-mental projections with the right side downwards, fluid level appears. All four projections from the same patient.

impossible to demonstrate fluid radiologically. In the remaining cases, i.e. those with mucous membrane thickening, using the four standard projections, secretion was only demonstrated in 24% of the cases positive at irrigation (Fig. 2). However, when the 5th projection was added, this figure rises to 88%, i.e. small amounts of secretion which are often concealed by the mucous membrane thickening are much more frequently diagnosed when this projection is added.

DISCUSSION

In an investigation of the present kind it is obviously essential to describe in detail the methods adopted. The physiological condition of the diseased sinus is not static and if the irrigation is to reflect the radiological picture with confidence, it is important that the interval between the radiological examination and the irrigation is short. In general, previous authors have not reported this interval. In the present investigation the patients returned for irrigation immediately after completion of the radiological examination.

From the clinical viewpoint, it is of interest to establish whether a sinus contains secretion or not. Secretion indicates impaired drainage with retention of liquid, usually infected, and consequently requires improved drainage and probably antibiotic treatment. If a radiological examination of the sinuses is available in the hospital this is a great aid in establishing the diagnosis of sinusitis. However, it is reasonable to demand that the radiological examination correlates with the sinus content with a high degree of confidence, particularly concerning the presence of liquid. The present investigation analysed the correlation between the irrigation and the radiological findings, particularly concerning fluid content in diseased sinuses. Secretion was demonstrated at irrigation in 86% of the radiologically completely opaque sinuses. This figure corresponds with that of previous

12 — No diagnosis of fluid on radiograms

64 % — Fluid demonstrated with 5th projection added

24 % — Fluid demonstrated with four standard projections

Fig 2 The frequency of radiologically demonstrated fluid in relation to secretion demonstrated by irrigation in maxillary sinuses with mucous membrane thickening.

authors (Ballantyne & Rowe 1949 Hinde, 1950; Vuorinen *et al.*, 1962 McNeill 1963)

In cases with mucous membrane thickening, a fluid level was demonstrated at the radiological standard examination in 15 %. At irrigation, however secretion was demonstrated in 66 %. The comparatively low figure of fluid demonstrated radiologically corresponds with the findings of some previous authors (Ballantyne & Rowe 1949 Hinde 1950) but is considerably lower than that demonstrated by others (Vuorinen *et al.* 1962 McNeill, 1963) The differences in the above figures may be explained by differences in mucosal thickening in different materials. The present investigation demonstrated that secretion is more frequent when there is a pronounced thickening than when there is a discrete mucous membrane thickening.

One of the aims of the present investigation was to improve the confidence of radiological demonstration of the presence of fluid and this was accomplished by the introduction of a 5th projection the occipito-mental with the patients head in a horizontal position and the affected side downwards. Using the four standard projections only 24 of the cases with secretion at irrigation could be demonstrated radiologically With the introduction of the 5th projection the radiological confidence in cases with mucous membrane thickening increases from 24 to 88 The complete radiological examination is only prolonged 3–4 min by the additional 5th projection Consequently this projection can be recommended for routine purposes in cases with mucous membrane thickening where the four standard projections fail to demonstrate fluid levels.

ZUSAMMENFASSUNG

Der Otologe hat das Recht, einen hohen Grad von Sicherheit bei der Röntgendiagnostik der Maxillarsinusitis mit oder ohne Exsudat zu fordern. Frühere Untersuchungen haben röntgenologische Veränderungen mit dem Punktionsbefund verglichen. Die hier vorgelegte Untersuchung berichtet über einen ähnlichen Vergleich, korreliert aber darüber hinaus die röntgenologisch demonstrierte Flüssigkeit mit der bei der Punktion nachgewiesenen.

Bei der Anwendung von vier Standardprojektionen fand sich Flüssigkeit bei der Punktion in 86% der gänzlich verschatteten Nebenhöhlen. In Fällen von röntgenologisch nachgewiesener Schleimhautverdickung wurde *Exsudat durch Punktion in 60% der* Fälle demonstriert, aber unter diesen Fällen wurde Flüssigkeit röntgenologisch nur in 24% nachgewiesen. Wenn eine fünfte Aufnahme hinzugefügt wurde in Form von occipito-mentaler Projektion mit dem Kopf seitlich geneigt und der affizierten Seite nach unten, stieg die Sicherheit der Röntgendiagnose hinsichtlich demonstrierter Flüssigkeit auf 88%. Diese Projektion wird empfohlen für die Routinediagnostik in Fällen, bei denen die vier Standardprojektionen Schleimhautschwellung aber keine Flüssigkeit zeigen.

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X RAY IRRADIATION OF THE INNER EAR OF THE GUINEA PIG

An Electron Microscopic Study of the Degenerating Vestibular Sensory Cell

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The inner ear of the guinea pig was irradiated with single doses of 7000 R X-rays. An electron microscopic study of the vestibular part of the labyrinth was performed after 1½, 3 4 and 6 hours. Changes were noticed chiefly in the type II but also in some type I sensory cells. The first changes were observed in the nuclei and consisted of clumping of the chromatin and the interchromatinic granules and swelling of the nuclear envelope. Later the nuclei became pycnotic. In the cytoplasm the early changes were: An appearance of large number of vacuoles with the surface studded with ribosomes, various mitochondrial alterations, slight increase in the number of lysosome-like bodies in the apical portion of the cells, and an appearance of sequesters of small cytoplasmic areas delimited by one or more layers of membranes. Severely altered cells appeared shrunken. The elimination of the degenerating cells is supposed to occur in two ways. The apical portion is expelled into the endolymph while the perinuclear portion is phagocytized by the supporting cells. Except for phagocytized material, no changes were noticed in the supporting cells.

In a light microscopic study of the vestibular part of the inner ear following irradiation with X-rays, degeneration of sensory cells was observed (Winther 1969 *a* and *b*). The degeneration was most pronounced in the periphery of the maculae and the cristae ampullares, and the degenerating cells seemed to be mainly of the sensory cell type II. The first detectable changes were found 3 hours after the irradiation and consisted of coarse granulation of the nuclei. Severely damaged sensory cells were found 18 hours following the irradiation.

The present investigation was undertaken to analyze the ultrastructural changes in the degenerating sensory cells, and to ascertain to what extent the degeneration of the vestibular sensory cells is comparable with that of the cochlear sensory cells under the same experimental conditions.

MATERIAL AND METHODS

Fourteen guinea pigs were irradiated with a single dose of 7000 R. They were sacrificed 1½ 3 4 and 6 hours after the irradiation. The source of radiation was a Siemens Stabilipan roentgen apparatus, operated at 290 kV 12 mA, and equipped with a Thoraeus filter II (0 8 mm Sn, 1.25 mm Cu, and 1 mm Al) The focus–skin distance was 232 mm The dose rate at the site of the inner ear was 200 R/min. The X-rays were delivered to the left half of the skull through a rectangular hole (20 mm × 17 mm) in a lead sheet (thickness 10.5 mm) which also shielded the rest of the animal. For details concerning the selection of the experimental animals, the irradiation conditions, and the dosimetry the reader is referred to a previous paper (Winther 1969 c)

After decapitation of the animals, the temporal bones were quickly removed from the skull, and the inner ears were opened Within 2–3 min of decapitation the labyrinth was irrigated with the fixative solution. The fixative mainly

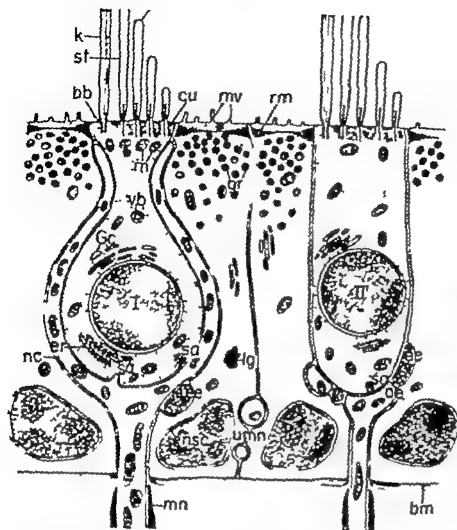


Fig. 1. Schematic drawing of the vestibular sensory and supporting cells (Courtesy of Lindeman, 1969). Sensory cell type I (*I*), sensory cell type II (*II*), kinocilium (*k*), stereocilia (*sf*), basal body (*bb*), cuticular plate (*cu*), microvilli (*mv*), reticular membrane (*rm*), mitochondria (*m*), multivesicular body (*vb*), Golgi complex (*Gc*), ergastoplasm (*er*), synaptic area (*sa*), nerve calyx (*nc*), efferent nerve ending (*ee*), afferent nerve ending (*ae*), nucleus of supporting cell (*nsc*), osmiophilic body (*lg*), granular body (*gr*), unmyelinated nerve fibre (*umn*), myelinated nerve fibre (*mn*), basal membrane (*bm*).

used was veronal-acetate buffered 1% osmium tetroxide at a temperature of 4 C Some specimens were fixed in cacodylate-buffered 2.5% glutaraldehyde and post-fixation was performed in veronal-acetate buffered 1.5% osmium tetroxide.

After partial dehydration in ethanol, the maculae and the cristae ampullares were dissected out. Dehydration was then completed and the specimens were embedded in Epon or Araldite Ultrathin sections were cut on an LKB Ultramicrotome, mounted on Formvar carbon coated copper grids and stained with lead citrate or with uranyl acetate followed by the lead citrate In all animals sensory areas from the left and the right side were prepared and examined in the same way The right side served as control.

RESULTS

Normal anatomy controls

Smith (1967) and Wersäll (1967) have given a detailed description of the submicroscopic struc



Fig. 2. Portion of the nucleus of normal type II sensory cell. Fixation/staining: osmium tetroxide/lead citrate. Chromatin (*Ch*). Nucleolus (*Nl*). The black arrow points to the small granules of interchromatinic substance. The white arrow indicates one of the larger nuclear granules. × 27,000.

ture of the vestibular sensory areas. In the present paper only a brief survey will therefore be given of the relevant ultrastructural details of the sensory cells as these appear in normal controls.

The sensory cells in the vestibular part of the labyrinth are located in the maculae sacculi and utriculi and in the cristae ampullares lateralis, superior and posterior. The sensory epithelia consist of sensory cells and supporting cells. There are two types of sensory cells (Wersäll, 1956; Engström & Wersäll, 1958). The type I and the type II sensory cells differ in their shape and nerve supply (Fig. 1). The type I cells are bottle-shaped. Their afferent nerve fibres constitute a calyx which covers most of the cell like a shell. The type II cells are cylindrical and are innervated by numerous afferent and efferent nerve endings of various size. The endolymphatic surface of the sensory cells, which bears one kinocilium and several stereocilia, is provided with a cuticular plate.

The internal structures of type I and type II cells are roughly similar. Quantitative differences are possibly present, but have not yet been analyzed with appropriate methods. The nucleus, which is situated in the basal third of the cell, contains heterochromatinic granules, interchromatinic material, and a nucleolus (Fig. 2). The heterochromatin is distributed all over the nucleus but is particularly concentrated in certain areas. The interchromatinic material is composed of a filamentous ground substance and smaller (80 A to 120 A) and larger (about 350 A) granules. The nucleolus, which is located in the periphery, is about 1 μ in diameter and composed of four parts as in other types of cells (Bernhard & Granboulan, 1968): the pars granulosa, the pars filamentosa, the pars amorpha and the nucleolar associated chromatin. The pars amorpha is inconspicuous.

The cytoplasm contains mitochondria, dense bodies, a few cisterns of granular endoplasmic reticulum, groups of ribosomes, a well developed Golgi apparatus, and a great number of vesicles. The latter are found evenly distributed in the cytoplasm. They are 400 A to 2000 A in diameter and may contain a slightly flocculent material. Ribosomes are observed on the surface of some of the vesicles. Some coated

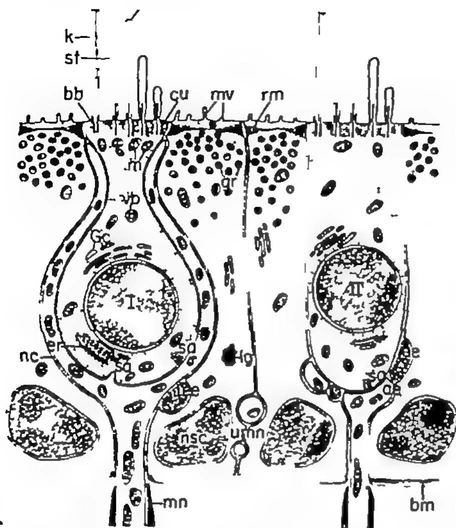


Fig 1 Schematic drawing of the vestibular sensory supporting cells (Courtesy of Lindeman, 1969). Sensory cell type I (*I*) sensory cell type II (*II*), kinocilium (*k*), stereocilia (*st*), basal body (*bb*) cuticular plate (*cu*), microvilli (*mv*), reticular membrane (*rm*), mitochondria (*m*) multivesicular body (*b*), Golgi complex (*Gc*), ergastoplasm (*er*), synaptic area (*sa*), nerve calyx (*nc*) efferent nerve ending (*ee*), afferent nerve ending (*ae*) nucleus of supporting cell (*nsc*), osmiophilic body (*le*), granular body (*gr*) unmyelinated nerve fibre (*umn*), myelinated nerve fibre (*mn*), basal membrane (*bm*).

used was veronal acetate buffered 1% osmium tetroxide at a temperature of 4 C Some specimens were fixed in cacodylate-buffered 2.5% glutaraldehyde and post-fixation was performed in veronal-acetate buffered 1.5 osmium tetroxide

After partial dehydration in ethanol, the maculae and the cristae ampullares were dissected out. Dehydration was then completed and the specimens were embedded in Epon or Araldite. Ultrathin sections were cut on an LKB Ultramicrotome, mounted on ... var carbon coated copper grids and stained with lead citrate or with uranyl acetate followed by the lead citrate In all animals sensory areas from the left and the right side were prepared and examined in the same way The right side served as control

RESULTS

Normal anatomy controls

Smith (1967) and Wersäll (1967) have given a detailed description of the submicroscopic struc



Fig. 4. Altered type II sensory cell 3 hours after the irradiation. Note the vacuoles (*V*) with the surface nearly covered by ribosomes. At arrow confluence between two vacuoles. The mitochondria (*m*) have a more globus appearance than in normal cells. The chromatin pattern is altered and the nuclear envelope is moderately dilated. The cytoplasm is bulging into the endolymphatic space (asterisk). Supporting cells (*Sc*). × 17,500. *Inset*: Areas of cytoplasm containing vesicles and ribosomes delimited by layers of membranes. × 35,200.

Dilatation of the nuclear envelope was a common feature. In some of the cells the dilated nuclear envelope formed sacs up to 3 to 4 μ in diameter. In addition, conspicuous changes were noticed in the cytoplasm (Fig. 4). The most impressive feature was the great number of vacuoles lined with a single membrane and with the surface studded with ribosomes. The



Fig 5 Degenerating vestibular sensory cell 6 hours after the irradiation. The nucleus (N) is pycnotic with an enormous dilatation of the nuclear envelope (asterisk). The cytoplasm is more electron dense than in normal cells and contains large number of vacuoles. Note the mitochondria containing electron-dense granules. A large portion of the cytoplasm protrudes at the endolymphatic surface 11,600.

vacuoles contained a slightly flocculent material and varied considerably in size The largest vacuoles were usually found near the nucleus. Confluence between the vacuoles was often seen In the apical portion of many cells there was a slight but nevertheless distinct increase in the number of dense bodies. Areas of cytoplasm-containing vesicles and ribosomes and delimited by one or more layers of membranes were usually encountered (Fig. 4 Inset) The mitochondria had a more globus configuration than in normal material. The internal structure of the organelles, however was apparently unaltered Some of the cells were swollen In these cells the cuticular plate was bulging toward the endolymphatic space and there was

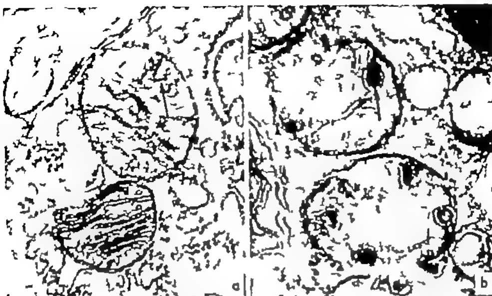


Fig 6 a, b Mitochondria from degenerating vestibular sensory cells 4 and 6 hours after the irradiation. Arrows point to needle-shaped (*a*) and spherical (*b*) inclusions closely related to the internal membranes. 60,000.

often rupture of the cell membrane at the endolymphatic surface not covered by the cuticular plate Through this rupture portions of the cytoplasm were expelled into the endolymph. As a rule the sensory hairs had a normal appearance, but in some instances giant, club-shaped hairs were seen

Stage III

The most conspicuous feature of this stage was shrinkage of the nucleus and the cytoplasm. The nuclear content was highly electron-dense. Granular electron-dense masses, presumably consisting of heterochromatin were sharply limited from lighter areas containing more distinct small granules, giving the nucleus a map-like appearance In spite of the shrinkage of the nucleus, large dilatations of the nuclear envelope were still present (Fig. 5) Also the cytoplasm displayed a high degree of electron-density The organelles observed in stage II were, however easily discerned. Whorls of myelin-like structures were also observed. The mitochondria were usually swollen owing to a pronounced dilatation of the inner chamber In some of these mitochondria electron-dense, needle-shaped inclusions were present. In others large granules were observed in close connection to the cristae. These granules often had a translucent core (Fig. 6 *a*, *b*) Protrusions into the endolymphatic space were observed more frequently and were larger than in the stage II (Fig. 5) The protrusions were usually composed of fine granular electron-dense masses and had an irregular outline At places their limiting membrane appeared interrupted. Some of the degenerating sensory cells presented constriction of the supranuclear region which gave them an hourglass configuration (Fig. 7) The internal structure of the nerve endings related to the altered cells was apparently normal. However nerve endings were seldom found in contact with degenerating sensory cells of the stage III. As a rule, nerve endings underlying degenerating cells were separated from these by long, slender processes of the supporting cells (Fig. 7)

Stage IV

This stage was characterized by the appearance within the supporting cells of large inclusion bodies of varying electron density and delimited



Fig 7 Degenerating vestibular sensory cell 3 hours after the irradiation. The cell has an hourglass configuration and the apical portion of the cytoplasm is protruding into the endolymphatic space The degenerating cell is almost completely engulfed by a supporting cell (Sc) which send out a slender leaf (asterisk) separating the sensory cell from nervous elements (N). 8 00.

by a double membrane (Figs. 8 9) Several of these inclusions had the appearance of degenerating sensory cells with the characteristics of stage III. Others displayed a nuclear and a cytoplasmic component but their nature could not be recognized because of advanced lytic alterations. Some of the inclusions appeared as large lysosomes with unidentifiable debris of varying electron density The nucleus and the cytoplasm of the supporting cells were otherwise normal

DISCUSSION

As in the outer hair cells of the organ of Corti, the first detectable changes in the vestibular



Fig 8 Cell displaying the typical features of degenerating sensory cell (*DS*) is engulfed by supporting cell (*Sc*). 12,000.

sensory cells after the irradiation were found in the nuclei and consisted of clumping and separation of the heterochromatin and the interchromatinic granules (Winther 1969 *d*) In the vestibular sensory cells, however the nuclear envelope was often dilated This phenomenon was not observed in the degenerating outer hair cells of the organ of Corti

Other differences in the mode of degeneration were noticed in the cytoplasm. In the outer hair cells of the organ of Corti early changes were marked hypertrophy of agranular endoplasmic reticulum and new formation of stacks of granular cisterns. One of the earliest changes in the cytoplasm of the vestibular sensory cells was the formation of vacuoles with the surface studded with ribosomes and containing some slightly flocculent material in an electron transparent matrix. It is probable that these vacuoles are formed from the granular vesicles and cisterns present in the normal sensory cells, since no increase in profiles of granular endoplasmic reticulum could be demonstrated. The large number of ribosomes on the surface of the vacuoles might be derived from the groups of free ribosomes. The electron-transparent appearance of the vacuolar content suggests that the swelling is due to an intake of water resulting from an alteration of the osmotic balance between the interior of the vacuoles and the surrounding cytoplasm. A dilatation of the nuclear envelope and the endoplasmic reticulum

verely damaged sensory cells This is in accordance with observations on streptomycin intoxicated animals (Wersäll & Hawkins, 1962 Spoendlin 1966) In many cases the nerve endings of both type I and type II sensory cells were separated from the degenerating cell by slender processes of the supporting cells. A similar phenomenon has been observed in the facial nucleus after section of the facial nerve (*Blinzinger & Kreutzberg, 1967 Torvik &* Skjörten, 1969) Slender processes of microglial cells extended along the surface and the dendrites of the degenerating nerve cells, thus separating the nerve cells from their boutons. Torvik & Skjörten (1969) showed that the degenerated neurons later were phagocytosed by the microglia.

In the last two decades the reaction of the vestibular sensory epithelia to streptomycin intoxication has been thoroughly investigated (Berg, 1951 Wersäll & Hawkins, 1962 Duvall & Wersäll, 1964 Spoendlin, 1966 Nagaba, 1968) It is therefore of interest to compare the sensory cell degeneration caused by streptomycin to that caused by the influence of X-rays. Although the pattern of degeneration with respect to sensory cell type and localization with- the sensory areas were different (Winther b) the acute cellular changes at the ultrastructural level following streptomycin intoxication had much in common with those observed after X ray irradiation. The changes described in the present paper are therefore not a specific effect of the X-rays

ZUSAMMENFASSUNG

Das innere Ohr von Meerschweinchen wurde mit Einzeldosen von 7000 R Röntgenstrahlen bestrahlt. Eine elektronmikroskopische Untersuchung uber den vestibulären Teil des inneren Ohres wurde nach 1½, 3 4 und 8 Stunden angestellt. Veränderungen wurden hauptsächlich in den Sinneszellen von *Type II* beobachtet, aber auch in einigen von Type I. Die ersten Veränderungen wurden in den Zellenkernen beobachtet und bestanden aus Zusammenballungen des Chromatins und der interchromatinischen Substanz. Ausserdem konnte auch ein Aufschwellen von der Kernmembrane wahrgenommen werden. Später wurden die Kerne pyknotisch Im Zytoplasma ergaben sich folgende frühen Veränderungen. Das Erscheinen einer grossen Anzahl von Vacuolen, deren Oberfläche mit Ribosomen übersät waren, verschiedene Veränderungen der Mitochondrien, eine leichte Vermehrung in der Anzahl von lysosomähnlichen Körpern im apikalen Teil der Zellen und das Erscheinen einer Sequestrierung on zytoplasmatischen Arealen, die von Membranen in einer oder uch mehreren Schichten begrenzt waren Stark veränderte Zellen schienen geschrumpft. Es wird angenommen, dass das Verschwinden der degenerierten Zellen in zwei Wegen hergehen. Der apikale Teil wird in die Endolymphe ausgestossen, *während der perinukläre Teil* von den Stütz-Zellen phagocytiert wird. Mit Ausnahme des phagocytierten Materials wurden keine Veränderungen in den Stütz-Zellen beobachtet.

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OTOSCLEROSIS

A Further Investigation of Inorganic Constituents by Neutron Activation Analysis

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The levels of isotopes of calcium, iodine, magnesium, copper chlorine, and sodium in stapedes and cortical bone from otosclerotic and non-otosclerotic patients were determined by neutron activation analysis. No significant differences were found in the levels of calcium, iodine, magnesium, chlorine, or sodium in otosclerotic stapedes or cortical bone when compared with the levels in the respective control tissues. Similarly no difference was found between copper levels in otosclerotic and normal stapedes.

a previous study we determined the con- of calcium and phosphorus in stapedes and in cortical bone from otosclerotic and non-otosclerotic patients by neutron activation analysis (*Solfer et al* 1969) No significant differences were found between otosclerotic and normal stapedes or between samples of cortical bone from otosclerotic and normal patients. These results conflict with that of a chemical analysis which indicated that calcium and phosphorus concentrations were lower in mallei, incudes, and stapedes from individuals with otosclerosis (Maurer 1961/62) Neutron activation analysis also indicated that there is no statistically significant correlation of calcium and phosphorus levels with age, sex or with the degree of otosclerotic growth.

Finally calcium to phosphorus ratios did not differ significantly among the tissues investigated by neutron activation analysis.

Although calcium and phosphorus levels showed no positive correlation with otosclerosis, the data obtained are valuable in that they provide *in situ* controls values for investigation of additional elements. This paper reports the results of such an extended investigation, i.e., neutron activation analysis of the levels of isotopes of calcium, iodine, magnesium, copper chlorine, and sodium in stapedes and cortical bone from otosclerotic and non-otosclerotic patients.

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MATERIALS AND METHODS

Stapedes and samples of cortical bone from the posterior meatal wall were obtained at surgery from patients undergoing stapedectomy for otosclerotic stapedial ankylosis. Control stapedes and cortical bone were obtained at autopsy from individuals free from gross liver kidney and ear pathology All samples were dried to constant weight and analyzed for inorganic constituents by neutron activation analysis. Irradiation and quantitation procedu-

Table 1 *Neutron activation analysis of stapedes and cortical bone*

mg: milligrams· µg: micrograms = 10^{-6} grams, ng: nanograms = 10^{-9} grams.

Sample	Sample weight (mg)	Ca (µg/mg)	I (ng/mg)	Mg (ng/mg)	Cu (ng/mg)	Cl (ng/mg)	Na (ng/mg)
Stapedes							
Normal							
401[a]	1.39	379	37	4 410	98	1 780	10,000
402	2.80	237	46	2,860	69	1,590	6,780
403	2.98	238	67	3,290	56	1,090	6,920
404	3.06	289	49	3,370	81	1,030	6,960
405	2.42	285	281	2,310	80	2,540	7,650
Average ± S.E.	2.53 ± 0.27	286 ± 23.12	49 7 ± 6.4	3,250 ± 346	76.8 ± 7.0	1,600 ± 274	7,660 ± 604
Stapedes							
Otosclerotic							
301[a]	3.55	239	34	3,610	104	3,970	10,800
302	3.19	271	68	2,560	67	4,030	8,440
303	3.25	297	70	3,070	88	2,640	9,030
304	3.52	320	62	3,020	67	1 760	7 730
305	2.48	246	66	3,910	67	1,070	7 430
Average ± S E.	3.20 ± 0 17	275 ± 13.64	60.0 ± 6.8	3,270 ± 232	78.6 ± 7.5	2,690 ± 588	8,690 ± 597
Cortical bone							
Normal							
401[a]	3 68	248	63	1,910	493[c]	1 650	7,360
402	4.97	257	45	2,710	182	2,400	8,090
403	6.23	296	49	2,820	161	1,610	7 430
404	2.94	341	48	3,140	117	1,040	8,510
405	3.70	295	216[c]	1,840	80	1,310	7,320
Average ± S.E.	4.30 ± 0.52	287 ± 14.80	51.2 ± 4.2	2,480 ± 259	135 ± 22.8	1,600 ± 228	7 730 ± 235
Cortical bone							
Otosclerotic							
301[a]	4.28	242	45	2,540	72	2,130	7,500
302	3.52	281	56	2,190	82	2,000	7,900
303	5.93	319	36	1 750	56	1,830	7 450
304	6.23	407	37	3,340	63	1,510	10,200
305	6.33	287	31	1,980	79	5,240	10,500
Average ± S.E.	5.25 ± 0.51	307 ± 24.87	41.0 ± 4 4	2,360 ± 277	70.4 ± 4 9	2,540 ± 682	8,710 ± 676

Corresponding numbers indicate samples from the same patient.
Standard error of the mean.
Value not used in mean.

res have been described in a previous paper (Soifer *et al* 1969) Because of the large number of isotopes included in this study a computerized program was used to calculate the results (Dooley *et al* 1968) The latter were expressed as micrograms of isotope per milligram dry weight of tissue.

RESULTS

The results of neutron activation analysis of stapedes and cortical bone appear in Table 1 *No significant differences were found in the* levels of calcium, iodine, magnesium, chlorine, or sodium in otosclerotic stapedes or cortical bone when compared with the levels in the respective control tissues. Similarly no difference was found between copper levels in otosclerotic and normal stapedes.

COMMENTS

The amounts of each of the elements of magnesium, copper chlorine, sodium, and io-

dine are the same in otosclerotic and non-otosclerotic bone tissue This observation points up the similarity in quantitative elemental composition between bone involved in otosclerosis, i.e., the stapes, and bone which is rarely involved, i.e., cortical bone. The higher amount of copper observed in normal cortical bone may be an exception but because of the rather wide range of values, we believe that little emphasis need be placed on this observation. Otosclerotic and control stapedes contain comparable levels of copper When DeJorge and co-workers (1965) determined serum copper in patients with otosclerosis, they found no abnormalities and concluded that copper metabolism is normal in otosclerosis. Recently Evans & Henkin (1969) reported a decrease in the total "mineral content" in one malleus and in incudes from otosclerotics as observed by photon beam absorption. These results support those of Maurer (1961/62) who found a decrease in the calcium and phosphorus content of endochondral bone from otosclerotics, and further support the premise that generalized changes occur in otosclerosis

Although stapedes were reported to be too ...ll to be measured by the photon beam tech... ue one would expect a similar decrease in otosclerotic stapedes. Yet such findings have not been demonstrated either by this or by previous activation analyses (Soifer *et al* 1969) Although the decrease could be attributable to an element yet undetermined by activation analysis, this seems highly unlikely Such an element would be present in such small quantities that it would be negligible compared with the large significant decrease observed by Evans & Henkin (1969) Perhaps there were no changes in the specimens of malleus and incus from the individual affected with chronic otitis media in addition to otosclerosis, but this was not discussed by Evans & Henkin (1969) Caution should be used when weighing results obtained from bone specimens from "chronic ears" as the ossicles are often structurally eroded in chronic ear disease

The values obtained for calcium are similar to those previously reported for other samples of stapes and cortical bone (Soifer *et al.*, 1969) When the results are expressed on the basis of *in situ* calcium content, the elemental analysis of otosclerotic and non-otosclerotic bone tissue remains the same Considering the similarity in elemental composition between involved and uninvolved bone and the fact that otosclerotic bone is histologically different, the implication is that there may be some difference in the structural relationship of the elements in otosclerotic bone. Another possibility is that despite the similar composition, there is a different turnover rate of the elements in otosclerotic bone resulting in a pathological condition.

The study of turnover rates certainly deserves attention, especially since few elemental compositional changes have been documented in otosclerosis.

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We should like to express our appreciation to Colonel Philip S. Gwynn, Director of the Nuclear Engineering Center for his consistent support of this research effort and to Mr John A. Dooley for donating his free time to assist us in adapting his computer code to solving the difficult analysis of a multispectra output. We also wish to thank Miss Kaarn Weaver and Mrs Sarah Thayer for their assistance in the preparation of this manuscript.

ZUSAMMENFASSUNG

Die Konzentrationen von Isotopen der Kalzium, Jod, Magnesium, Kupfer Chlor und Natrium in Steigbügeln und in Knochenteilchen von proximalen Ende der hinteren Gehörgangswand von Patienten mit und ohne klinische Otosklerose wurden mit Neutron Aktivation Analyse untersucht. Keine Unterschiede in Konzentrationen der Kalzium, Jod, Magnesium, Chlor und Natrium in Steigbügeln und in Teilchen der knöchernen Gehörgangswand wurden zwischen Otosklerosepatienten und Patienten ohne Otosklerose gefunden. Ebenso wurde kein Unterschied zwischen den Konzentrationen der Kupfer in otosklerotischen und normalen Steigbügeln gefunden.

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PRESBYACUSIS

III Perstimulatory Threshold Adaptation

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Studies on perstimulatory threshold adaptation were made in 41 presbyacutic ears with a Grason-Stadler model E 800 Békésy-audiometer using both interrupted and continuous test tones. No adaptation appeared when 200 msec pulsed tones were used. With continuous tones adaptation after 3 min was always less than 10 dB at 250 and 500 Hz. At 1,000 Hz adaptation between 10–30 dB was found in 8% at 2,000 Hz in 25% and at 4,000 Hz in 29% During the stimulation, the excursion amplitude remained unchanged with a pulsed tone and with continuous tone there was diminution in size at 4 000 Hz only

an earlier work (Jokinen, 1969) two control groups and two presbyacusis groups were used for studies of hearing threshold by manual and automatic audiometry One control group consisted of experienced, the other of inexperienced listeners, while the groups of presbyacusis consisted of pure presbyacusis and of cases associated with a C5 dip The manual thresholds were poorer than the automatic thresholds in all groups except the experienced control group Comparison of pulsed tone (200 msec) and continuous tone automatic audiometry thresholds showed no difference between the control groups, but in both groups of old people the pulsed tone gave clearly better thresholds. Also the threshold amplitudes were significantly larger in the aged than in the control groups while the size of the excursions became smaller towards higher frequencies in all groups

Another study (Jokinen, 1970) dealt with the effect of tone duration and intensity change on the hearing threshold in presbyacusis With an intensity change of 2.5 and 5 dB/sec and tone duration of 170 500 and 1,300 msec, no significant differences were found in the hearing thresholds Thus a pulsed tone of 170 msec duration was sufficient also in presbyacusis for a full loudness value. On the other hand, short tone auditory adaptation seemed unlikely since the thresholds with 1,300 msec tones were not affected. Thus the interruption procedure as such may be responsible for the better thresholds obtained with pulsed tones as compared with continuous tones.

In the present study the development of perstimulatory threshold adaptation in presbyacusis is analyzed during a 3-min time period. By so doing, the amount of adaptation at various frequencies can be accurately measured and further light may be shed upon the differences between the hearing thresholds for interrupted and for continuous tones.

MATERIAL AND METHODS

The group under study consisted of 23 persons suffering from pure presbyacusis (41 ears) with no history of previous ear diseases or any significant noise exposure. The age of the patients

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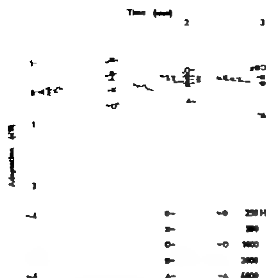


Fig. 1. Adaptation at various frequencies as a function of time. Interrupted tone, 200 msec pulses with 200 msec free interval.

varied from 59 to 86 years (average 72 years) only 3 subjects being under 65 years of age.

The thresholds were first recorded manually using the Madsen model OB 60 audiometer. A Grason-Stadler model E 800 automatic audiometer was then employed using the frequencies 4 000, 2,000 1 000 500 and 250 Hz in the order mentioned. For each frequency the subject first recorded his thresholds for a pulsed tone and then for a continuous tone during a period of 3 min. The method of adjustment was used, the subject pressing the key as long as the tone was heard and releasing it as soon as the tone disappeared. The rate of intensity change for both the pulsed and the continuous tones was 2.1 dB/sec in 0.25 dB steps. The duration of the tone pulse was 200 msec with an on-off period of 50%.

From the adaptation records, the maximal and minimal values of the excursions were tabulated from a period of 20 sec at the start of the test and at 1 2 and at 3 min. The hearing threshold at any of these periods was then calculated as a mid-point value. Excursion amplitudes were tabulated from the same time periods as the differences between the maximum and minimum values. The amount of possible adaptation could then be easily calculated as the difference between the hearing thresholds as a function of time. The data were handled at the Computer centre of Oulu University. The student's *t*-test was used for calculating the significance of the differences, and the level of $P < 0.01$ was applied as indicating significant differences between two observed values.

RESULTS

The results using the pulsed tone during the 3-min perstimulatory test are seen in Fig. 1. The amount of adaptation at any recorded moment was found to be minimal and always less than 1 dB. No significant differences from zero-line, or between any individual frequencies, were noted at any given point.

Fig. 2 shows the same test results by using continuous tone. The results for 250 and 500 Hz, showing no adaptation, do not differ sig-

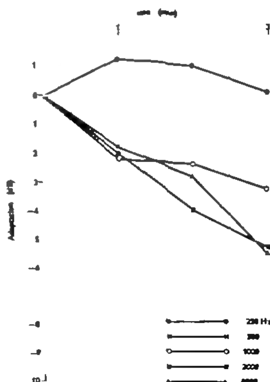


Fig. 2. Adaptation at various frequencies as a function of time. Continuous tone.

nificantly from each other or from the expected zero-line at any point. The figures 1 000, 2,000 and 4 000 Hz, which show varying amounts of adaptation, do not differ statistically from each other at the points measured. This is the case even though the 3-min value for 1 000 Hz is clearly smaller than those for 2,000 and 4 000 Hz.

Adaptation at 1 000 Hz was significantly larger ($P<0\ 01$) than at 250 Hz at all recorded points. The difference between 250 and 2,000 Hz was highly significant ($P<0\ 001$) and a difference of this amount also occurred between 250 and 4 000 Hz at the 3-min point. At the 1 and 2-min points the differences were significant ($P<0\ 01$) between 250 and 4 000 Hz.

Between 500 and 1 000 Hz there were no statistically significant differences although the slopes of the curves were different. Between 500 and 2,000 Hz there was a highly significant difference at the 2 and 3-min points and between 500 and 4 000 Hz at the 3-min point.

The growth of adaptation as a function of time can also be seen from Fig. 2. During the first minute, adaptation at 1 000, 2,000 and 000 Hz was about 2 dB For 2,000 Hz this end continued during the whole stimulation period, whereas for 4 000 Hz the average at 2 min was slightly less though the end result was the same. For 1 000 Hz the whole curve took a more gradual slope after the first minute With the pulsed tone there were no differences between various frequencies and no adaptation.

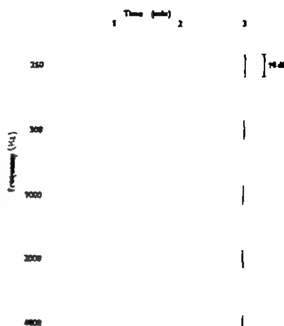


Fig 3 Excursion amplitudes at various frequencies as a function of time. Continuous tone.

Using the pulsed test tone, the width of the amplitude remained unaltered during the whole 3-min testing time Using the continuous tone (Fig. 3) there was a slight decrease during the 3-min period, but only at 4 000 Hz was the difference (1.3 dB) statistically significant ($P<0\ 01$) The amplitudes decreased as a function of frequency towards the high tones.

The hearing levels with the pulsed tone were

Table 1 *Comparison of average hearing thresholds with pulsed and continuous tones*

	Time (min)							
	0.3		1		2		3	
Frequency	T.D	S.D	T.D	S.D	T.D	S.D	T.D.	S.D
250	2.6	4 7	2.4	5.3	1 6	4 6	2.4	4 2
500	2.0	3.8	2.1	4 1	2.8	4.8	2.0	4 5
1,000	2.6	5 9	4.5	7 4	6.0	8.3	6.9	9 1
2,000	4.8	5.9	7 6	8.7	9 6	11 6	11 0*	12 9
4,000	5.8	7 3	8.2	9 4	8.3	9 6	10 7	11 5

T.D = Threshold difference
Degree of significance: $P<0.01$ $P<0.001$

Table 2. *Individual classification of 3-min adaptation values*

	Adaptation (dB)					
	Pulsed tone			Continuous tone		
Frequency	0–10	11 30	over 30	0–10	11 30	over 30
250	37	0	0	36	0	0
500	37	0	0	39	0	0
1,000	40	0	0	36	3	0
2,000	38	0	0	32	8	0
4,000	38	0	0	31	9	0

at all points significantly better than those with continuous tone (Table 1) This was noted even at the first point measured (20 sec) The width of the average excursions, on the other hand, showed no significant differences.

Table 2 shows the individual classification of the adaptation values at the 3-min points in this material. At 250 and 500 Hz all values were less than 10 dB, while values between 11 and 30 dB occurred at 1 000 Hz in 8.3% at 2,000 Hz in 25% and at 4 000 Hz in 29% All other values for these frequencies were less than 10 dB Adaptation never exceeded 30 dB and in none did a total disappearance of the tone occur With the pulsed tone, all adaptation values were less than 10 dB

DISCUSSION

In normally hearing ears, automatic threshold recording at a fixed frequency with an interrupted test tone results in straight line tracings without adaptation. This is also generally the case in various hearing disorders, except some cases of VIII nerve pathology particularly acoustic tumours, in which even interrupted tone tracings with short off-times may show some adaptation (Jerger & Jerger 1966) In presbyacusis the interrupted 3-min fixed frequency tracings never showed any adaptation. An off-time of 200 msec obviously is sufficient for a full recovery

As regards adaptation as tested with continuous tones in presbyacusis, the present results agree reasonably well with the data reported by Goetzinger *et al* (1961) Jerger (1960) Schindler (1962) Palva *et al* (1967) and Gjevenes & Söhoel (1969) As a rule, there is very seldom adaptation exceeding 30 dB in presbyacusis. In the present study none of the values exceeded 30 dB, which may be due to the fact that the manual method used by the earlier investigators, is more sensitive than the automatic threshold recording technique (Palva *et al* 1967)

While in the study of Goetzinger *et al* (1961) the frequencies 500, 1 000 and 2,000 Hz were tested Gjevenes & Söhoel s (1969) covered 500 2,000 and 4 000 Hz. The finding made in both of these studies, viz. that larger amounts of adaptation are found at high frequencies, is confirmed by the present results. Furthermore, using automatic recording, the present results show that for the frequencies 250 and 500 Hz adaptation is always less than 10 dB At 2,000 and 4 000 Hz adaptation between 11 and 30 dB appears in roughly 25% of the cases but at 1 000 Hz in only 8% Thus correlation of adaptation values with clinical groups would probably profit from comparison of adaptation recordings at 500 and 2,000 Hz. If considerable adaptation is demonstrated at 500 Hz, retrocochlear nerve fibre pathology should be thought of (Gjevenes & Söhoel, 1969)

The amplitude of the threshold excursions diminished very little as a function of time. The average threshold amplitudes from 7 to 10 dB were all of the same order as in normal or non-recruiting ears (Palva, 1957) The fact that the threshold tracing became only slightly smaller as a function of time correlates well with the nonrecruiting nature of presbyacusis

Study of the various subdivisions by age shows that, in this series of presbyacusis, the adaptation values were not distinctly affected by age Adaptation does not explain the differences between pulsed and continuous tone hearing thresholds in old people since better thresholds for pulsed tones at all recorded points were always recorded both at frequencies showing adaptation and at those show-

ing no adaptation. It appears likely that the interruption procedure itself is responsible for the better thresholds obtained with pulsed tones.

ZUSAMMENFASSUNG

Untersuchungen über die perstimulatorische Adaptation an der Gehörschwelle wurden bei 41 altersschwerhörigen Ohren mit einem Grason-Stadler Model E 800 Békésy Audiometer unter Verwendung eines pulsierenden sowie eines Dauertons durchgeführt. Keine Adaptation trat auf, wenn ein pulsierender Ton von 200 msec verwendet wurde. Bei Dauertönen war die Adaptation nach 3 Minuten immer geringer als 10 dB bei 250 und 500 Hz. Bei 1 000 Hz wurde Adaptation festgestellt zwischen 10–30 dB bei 8% bei 2 000 Hz bei 25% und bei 4 000 Hz bei 29% Während der Stimulation blieb die Amplitude unveränderlich bei pulsierendem Ton und bei Dauerton wurde eine Verringerung nur bei 4 000 Hz festgestellt.

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PATTERNS OF PURE TONE HEARING LOSS

A Comparative Study of Presbycusis Multiple Sclerosis Menière's and Acoustic Neuroma

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A comparative study has been carried out of the incidence of the specific patterns of hearing loss in subjects with presbycusis, multiple sclerosis, acoustic neuroma and Menière disease. The descending type of pattern was most frequently seen with almost the same incidence in presbycusis, multiple sclerosis and acoustic neuromas. The flat losses were most frequently seen in Menière's disease. These seemed to be comparable to the flat loss in cases of metabolic presbycusis described by Schuknecht. The low tone losses were almost exclusively seen in Menière's disease. A suggestion has been made that the descending audiometric curve in presbycusis may possibly be explained by an involvement of the neural elements based on the finding of a similar frequency of descending audiogram in multiple sclerosis and acoustic neuroma.

Patterns of pure tone hearing loss have been discussed in a number of papers dealing individually with the various disease entities (Dayal & Swisher 1967 Dayal *et al.*, 1966 Johnson, 1968 Enander & Stahle, 1967). However a comparative study of these diseases with regard to the patterns of hearing loss is not available. For the present study therefore we have chosen to deal with the comparison of various patterns of pure tone hearing losses as seen in Menière's disease acoustic neuroma, multiple sclerosis and presbycusis.

Most subjects in this study have been subjected to an extensive audiometric investigation including pure tone thresholds, speech reception thresholds, discrimination for speech, SISI, ABLB tone decay and Békésy audiometry However only the pure tone patterns have been considered in this analysis.

SUBJECTS

For the presbycusis group, 55 consecutive records of all patients with a clinical diagnosis of presbycusis were used. The average age of this group was 65 The range being 42 to 82 years. Only patients with bilateral symmetrical sensorineural hearing losses were used in this study Patients with asymmetrical curves between the two ears or with conductive hearing loss were not included. Any patient in whom the hearing loss could be related to any other factor viz. stimulation deafness etc. was not included in this study All subjects were seen at the Toronto General Hospital.

The subjects in the multiple sclerosis group are from a previous paper of one of the authors (Dayal *et al.*, 1966)

Fifty-six patients with Menière's disease were taken from the files at the Toronto General Hospital. Only cases with a definite diagnosis based on audiograms, history and vestibular examination were included. Cases in which the diagnosis was doubtful were eliminated.

Twenty-six patients with acoustic neuroma were cases seen at the Toronto General Hospital

and the Royal Victoria Hospital, Montreal. All cases were either confirmed by a tissue diagnosis or a posterior fossa contrast X-ray study In all cases the subjects chosen were the result of random selection.

EQUIPMENT

Tests were done in a soundproof room with Maico, MA8A or MA24 audiometers, routinely calibrated prior to examination. A conventional audiometric technique was used to determine the pure tone thresholds.

Classification of audiograms with hearing losses was similar to that described by Carhart (1945) with some modification. The classification of the patterns was primarily based on the general configuration of the audiometric curve

The "flat group" consisted of sensorineural losses at all frequencies, but not differing by more than 10 dB in unsystematic fashion between two different frequencies, emphasis being placed in the speech range. "Low tone groups" were those with a loss of at least 10 dB or more at lower frequencies, but with better thresholds at higher frequencies. Descending curves were by losses greater than 10 dB in frequencies, over a range inclusive of or more octaves. The dome-shaped group demonstrated pure tone losses of at least 10 dB at both low and high frequencies, with better function in the middle range. A small number of unclassifiable curves were grouped under "others"

FINDINGS AND DISCUSSION

Most of the patterns of hearing loss were easily classified under the four major groups.

Table 1 shows the relative frequency with which the descending and flat curves are seen in presbycusis, multiple sclerosis, acoustic neuroma and Menière s disease. For the statistical analysis a series of chi square tests was performed.

The descending curve is seen with almost the same frequency in presbycusis as in multiple sclerosis and acoustic neuroma patients. The percentages for the three groups are 65% 67% and 65% respectively Again the flat losses have similar incidence in presbycusis, multiple sclerosis and acoustic neuromas (Table 1).

Table 1

	Presby-cusis	M.S.	Ac.N	Menière s
Descending	36 (65 %)	16 (67 %)	17 (65 %)	5 (9 %)
Flat	17 (31 %)	4 (17 %)	8 (31 %)	31 (55)
Low tone	0 (0 %)	1 (4 %)	0 (0 %)	14 (25 %)
Dome	0 (0 %)	0 (0 %)	[illegible] (0 %)	5 (9 %)
Others	2 (4 %)	3 (12 %)	1 (4 %)	1 (2 %)

Number in brackets indicates percentage incidence.

However in Menière s disease the patterns are quite different from the previous three groups ($p < 0.01$) in that the descending curves are only seen in 9% flat in 55% and the low tones in 25% The low tone losses were almost confined to the Menière s disease group only

Even though the number of patients in this study is not very large, the results seem to be of significance as they compare closely to results of larger series. In a large group of acoustic neuroma patients Johnson (1968) reported a 64% incidence in the descending type and 20% incidence in the flat loss. These figures correspond closely to the figures obtained from our group of acoustic neuroma cases where the descending type of patterns are seen in 65% and the flat patterns in 31% of the patients.

In the Menière s disease group the percentage incidence of patterns are very similar to those obtained by Enander & Stahle (1967). They found an incidence of 60% of flat curves, 17% of rising curves and 12% of falling curves. Our studies show in the Menière s group, 55% with flat patterns, 25% with low tone or the rising curve and 9% in the descending or the falling curve.

It is interesting to correlate these patterns to the site of pathological involvement in the four groups of subjects. Multiple sclerosis affects the auditory and vestibular systems in various ways. However the lesion in this disease is

located central to the neurolemmal-neuroglial junction. The pure tone losses seen in these patients represent a neural type of disorder. In other words involvement of the eighth nerve itself. In a small percentage of patients with multiple sclerosis the ageing process may have added its effects to the pattern of the disease. However, in a previous study (Dayal & Swisher 1967) it was noted that in the multiple sclerosis group 59.1% of the patients had a pure tone loss as compared to 27.3% in a closely matched control group. It was concluded at that time that "there is a small and definite number in the multiple sclerosis group where the pure tone hearing loss is secondary to the demyelinating process".

The pathological changes in acoustic neuroma is located in the eighth nerve i.e. neural elements. However, in 20% of these patients a predominantly end organ type of hearing loss is seen. This has been attributed to vascular compression affecting the nutrition of the hair cells.

The pathology in Menière's manifests itself primarily as an end organ type of hearing loss. Presbycusis as defined and described by Schuknecht (1967) consists of basically the sensory, the neural, the metabolic and the mechanical types of pathology. The four disease entities studied in this investigation, therefore provide excellent material for a comparison of manifestations belonging to Schuknecht's four types and our material representing two different pathologic localizations. (1) primarily the inner ear as in Menière's, and (2) the eighth nerve as in multiple sclerosis and in acoustic neuromas.

Our data show a significantly similar incidence of the patterns of losses in multiple sclerosis and acoustic neuromas, the most frequent being in the descending curve and the flat patterns being the next most common. In multiple sclerosis and acoustic neuromas pure tone losses represent eighth nerve involvement, i.e. pathology in the neural element. It therefore does not seem unlikely and it is very tempting to suggest that the cases with descending pattern seen with similar frequency in presbycusis as in multiple sclerosis and acoustic neuromas might indicate a neural type of pathology. The site of pathology of the descending pattern in presbycusis is still not clearly understood (Schuknecht, 1967).

As earlier mentioned, the percentages of both descending and flat audiograms in acoustic neuroma, multiple sclerosis and presbycusis are similar in these three categories. The patients with Menière's disease on the other hand show a very different pattern of hearing loss, i.e. predominantly presenting a flat audiogram and some with low tone loss.

It is interesting to compare the hearing loss in Menière's disease with the audiometric findings in the metabolic type of presbycusis described by Schuknecht (1967). Even if these two processes are aetiologically different, it is interesting to notice the similarity in the pattern of their hearing disturbance. He described the flat type of hearing loss in the metabolic group of presbycusis. This same type is also seen in our series of Menière's patients as the most frequent change recorded. The pathologic changes in Menière's disease are due to some metabolic disturbances affecting the peripheral end organs.

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ZUSAMMENFASSUNG

Eine Untersuchung von dem prozentuellen Vorkommen von charakteristischen Kennzeichen der Reinton-Audiogramme in Fällen von Presbyacusis, Multiple Sklerose, Acusticus Neurinoma und Menièresche Krankheit ist vorgenommen worden. In Fällen mit Presbyacusis, Multiple Sklerose und Acusticus Neurinoma wurde eine abfallende Kurve des Audiograms öfters und mit fast derselben Frequenz in diesen drei Gruppen gefunden. Ein Gehörverlust im Tief Ton-Gebiet wurde fast ausschliesslich bei Menière-Patienten gefunden. Meistens wurde aber eine flache Kurve in der Menièreschen Krankheit gefunden. Diese Typen der Audiogramme gaben den Anschein mit den Kur-

ven Schuknecht's zu Übereinstimmen die als Merkmal einer metabolisch verursachten Presbyacusis beschrieben wurden. Die abfallende Kurve der Audiogramme in Fällen von Presbyacusis macht es naheliegend anzunehmen dass auch in diesen Fällen, wie in Fällen von Multipler Sklerose und Acusticus Neurinoma die Veränderung in dem nervösen Anteil liegen.

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QUANTITATIVE ANALYSIS OF ACID MUCOPOLYSACCHARIDES IN THE NORMAL GUINEA PIG COCHLEA

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The turbidimetric method based on the formation of insoluble complexes between acid mucopolysaccharides and cetyltrimethylammonium bromide was modified for micro-analysis. The distribution of acid mucopolysaccharides in the membranous cochlea of the guinea pig was measured using this technique combined with enzymic analysis. Both the tectorial membrane and Reissner's membrane contained 0.1% of acid mucopolysaccharides per dry weight. Both the stria vascularis with spiral ligament and the basal membrane with the organ of Corti and limbus spiralis contained about 0.6%. The mean dry weight of different parts of the membranous cochleae was obtained for reference by the use of quartz helical balance. The tectorial membrane weighed 4.6 μg per cochlea.

Interest in the tectoral membrane has been centered on its possible polarizing role between the positive (ca +80 mv) potential in the endolymph and the negative (ca −80 mv) potential in the organ of Corti (Naftalin, 1965 Saito, 1967). Lawrence (1967) also suggested polarization of the tectorial membrane as a result of demonstrating its zero potential.

Further the presence of acid mucopolysaccharides in the membrane would be highly significant since they would be capable of polarizing it. Many attempts have been made to identify the chemical composition, especially of acid mucopolysaccharides, of the tectorial membrane. The results obtained by various workers were not always in agreement (Iurato, 1960) Recent work, (Iurato, 1960 Naftalin *et al.* 1964) however showed that there were no acid mucopolysaccharides in the tectorial membrane, although they might be present in such small quantities as not to be detectable by the methods employed. Therefore, it is still uncertain as to whether they exist, or not, in the tectorial membrane.

On the other hand, the progress of connective tissue biochemistry revealed the mucopolysaccharidoses, a group of diseases with widespread manifestations in the connective tissues (Schubert & Hamerman, 1968) It is of great interest that deafness is one of the symptoms of Hurler's syndrome, which is one of the mucopolysaccharidoses It has been reported that the patients with Hurler's syndrome increase urinary excretion of chondroitin sulfate B and heparitin sulfate This excretion is related to the excessive formation and storage of these polysaccharides in patients organs. However little is known about the etiology of the mucopolysaccharidoses. It is, therefore, worth studying the distribution of acid mucopolysaccharides in the inner ear since deafness may be due to the mucopolysaccharidoses

This study was designed to demonstrate the existence of acid mucopolysaccharides in the tectorial membrane and their distribution in the inner ear

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ren Schuknecht s zu /bereinstimmen die als Merkmal einer metabolisch verursachten Presbyacusis beschrieben wurden. Die abfallende Kurve der Audiogramme in Fällen von Presbyacusis macht es naheliegend anzunehmen dass auch in diesen Fällen, wie in Fällen von Multipler Sklerose und Acusticus Neurinoma die Veränderung in dem nervösen Anteil liegen.

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75 μg of standard ChS-A was completely decomposed.

Other reagents used were of reagent grade.

Extraction of acid mucopolysaccharides from tissue

After measuring dry weight each tissue was homogenized with 2.0 ml of 0.01 M Tris-HCl buffer pH 7.5 by the use of a microhomogenizer. To the homogenates 0.2 ml of 5% Pronase was then added, which were incubated at 45 C for 5 hours to release AMPS from their protein bonds.

To remove proteins and nucleic acids, 1.8 ml of 22.2% trichloroacetic acid (TCA) solution (final concentration 10%) was added. The final volume was 4.0 ml. The interfering reaction of nucleic acids will be discussed later. After allowing the final volume to stand for one hour at room temperature, the precipitate was removed by centrifugation. The supernatant was decanted into another tube with a fine pipette. To the supernatant, 16 ml of ice chilled absolute ethanol followed by 0.2 ml of 25% potassium acetate to precipitate AMPS was added (Meyer *et al.*, 1956). After mixing thoroughly the tubes were chilled in ice over night. To the precipitation centrifuged by 1700 × G for 80 min, 2.0 ml of distilled water was added. This solution including AMPS was dialyzed against distilled water with cellulose tubing, obtained from the Thomas Company (the average pore diameter was 48 Angström units) for over 4 hours. To the dialyzate plus 0.5 ml of distilled water which was used to rinse the cellulose tubing, 0.12 ml of 25% potassium acetate and 15.0 ml of ice chilled absolute ethanol were added. After mixing thoroughly the whole was chilled in ice over night.

The reaction tube was again centrifuged by 1700 × G for 80 min. The precipitation, containing pure AMPS was dissolved in acetate buffer from 0.5 to 5.0 ml according to the amount. This solution was used for turbidimetric measurement of AMPS.

Measurement of acid mucopolysaccharides

Quantitative measurement was done by the turbidimetric method based on the formation of relatively insoluble complexes between isolated AMPS and CTAB. It was found by Di-Ferrante (1956) that the amount of turbidity developed when CTAB is added to a solution of AMPS is proportional to the amount of AMPS in the system. This method was modified for micro-analysis in the present study. The modification will be discussed below.

Into each test tube 1 ml of acetate buffer containing from 1 to 100 μg of AMPS was placed. In another tube 1 ml of acetate buffer was used as a blank. The tubes were immersed in a water bath maintained at 30 C. After 10 min, one ml of CTAB-Li reagent was added to each test tube and mixed. It was noteworthy that the pH was 12.5 at this step. After incubation at 30 C for 5 min, 0.2 ml of 2 M NaCl was added to each tube. After mixing and incubation at 30 C for another 15 min, the content of each tube was transferred to a cuvette (0.9 ml capacity) and the optical density (O.D.) of each sample was read against the blank. It took at least 30 min for the total procedure. A Beckman DU Spectrophotometer was employed in this work, and readings were taken at $\lambda = 400$ mμ with a slit width of 0.07 mm. Fig. 1 shows the standard calibration curve with ChS-A which was previously prepared. A good proportional relation between the turbidity and the amount of ChS-A was obtained from 1 to 40 μg per ml. Maximum sensitivity was 1 μg per ml or 0.5 μg per 0.5 ml.

The acetate buffer which passed through the same procedure with tissue was used as a blank to check the turbidity of the process. A recovery test showed from 93% to 98% recovered with a fine pipette and refined technique. Dilution or concentration of the solution was made according to the amount of AMPS extracted. The concentration of AMPS in the solution was then obtained from the calibration curve, and the total amount was calculated.

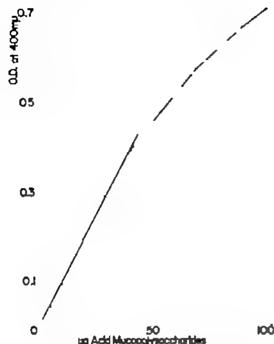


Fig 1 The relation between the turbidity as measured at 400 mμ and the amount of sodium chondroitin sulfate A per ml. Standard calibration curve

The specificity of this method for AMPS has been tested by DiFerrante (1956) According to his data, the turbidity of AMPS given 100 μg showed that sodium chondroitin sulfate was 133% sodium heparinate 143% as sodium hyaluronate 100% while other compounds were almost negligible In the present study ChS-A was adopted as a standard. Its turbidity was not equal to other kinds of AMPS However this method was very useful to measure the total amount of AMPS

The preliminary test of the complex formation with pronase and formalin under this method showed no turbidity However deoxyribonucleic acid (DNA) showed about 92% of turbidity compared with ChS-A as 100% while ribonucleic acid (RNA) was negligible. This was the reason why TCA solution was used in the procedure

Enzymic analysis of acid mucopolysaccharides
Although CTAB reagent showed good specificity for AMPS, nucleic acid, especially DNA, interfered with the assay (Kawamoto *et al.*, 1968) TCA solution was used to remove them but there was still a possibility that some remained.

The contamination with nucleic acids was first checked with the absorption at 260 mμ in the ultraviolet region, compared with the standard calibration curve previously prepared. DNase was then used to decompose the rest of the DNA. The reaction of DNase with CTAB-Li reagent was examined. Complex formation or interference was not observed at a concentration up to 2000 Kunitz units of DNase per ml. It has been reported that 100 Kunitz units were sufficient to remove DNA, and that almost no decomposition of all kinds of AMPS was observed (Kawamoto *et al.*, 1968)

The turbidity formation by CTAB reagent indicates the whole amount of all kinds of AMPS Therefore, testicular HAase which decomposes chondroitin (Ch) ChS-A, ChS-C and hyaluronic acid (HA) and CHase ABC (Yamagata *et al.*, 1968) which decomposes Ch, ChS-A, ChS-B Ch-C and HA were employed to analyse AMPS The combination of these enzymic decompositions evaluated HAase digestive AMPS, HAase resistant AMPS and ChS-B

The procedure was as follows. 0.5 ml of acetate buffer containing AMPS extracted from tissues was delivered into 4 test tubes. To the first tube (*a*) 0.5 ml of acetate buffer was added to make the volume one ml. To the second tube (*b*) 1000 Kunitz units of DNase in 0.5 ml of acetate buffer was added To the third tube (*c*) 250 T.R.U of HAase in 0.5 ml of acetate buffer was added. To the fourth tube (*d*) 1.25 units of CHase ABC in 0.25 ml of 0.25 M Tris-HCl buffer and 125 T.R.U of HAase in 0.25 ml of acetate buffer were added. Blank tubes for each enzyme were prepared using 0.5 ml of acetate buffer instead of the solution extracted from tissues. The final incubation volume was 1.0 ml. The tubes were incubated at 37 C for 20 hours, and the reaction was stopped by adding 1 ml of CTAB-Li

reagent. Measurements were made as described previously

Each turbidity was corrected by the blank. The difference of turbidity between (*a*) and (*b*) showed the contamination of DNA, and the turbidity of (*b*) was considered the amount of genuine AMPS The difference between (*a*) and (*c*) indicated HAase digestive AMPS such as HA, Ch, ChS-A and ChS-C. The difference between (*c*) and (*d*) indicated the amount of ChS-B The remaining turbidity of (*d*) indicated the other HAase resistant AMPS such as keratosulfate or herparin and contamination of DNA. Therefore, the difference between the turbidity of (*d*) and of DNA showed the other HAase resistant AMPS except ChS-B (Fig. 2)

RESULTS

1 *Dry weight of the membranous cochlea of the guinea pig*

The mean value of the dry weight of the membranous cochlea was obtained to insure the technique of dissection and for reference. The cochleae which failed to separate completely were excluded from the calculation. The results showed that the tectorial membrane weight per cochlea was 4 6 μg; the basal membrane with the organ of Corti and limbus spiralis 60.3 μg; and the stria vascularis with the spiral ligament 230 0 μg. The total dry weight of one membranous cochlea was 293 9 μg. Reissner's membrane was so light that it was considered to be included within the range of error The small deviation proved the satisfactory separation of the cochlea. The details are shown in Table 1

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Body weight from 250 to 350 g

Parts of cochlea	Series	Nos. of cochlea	Total D W (μg)	D W cochlea (μg)
Tectorial membrane	A	80	397.5	5.0
	B	150	657.9	4 4
	C	220	1,030.0	4 7
	Total	450	2,085 4	4.6
Basal membrane with organ of Corti & limbus spiralis	A	59	2,927 4	49 6
	B	140	8,347 7	59.6
	C	219	13 914 1	63.5
	Total	418	25 189.2	60.3
Stria vascularis with spiral ligament	A	84	20,128.2	239 6
	B	150	34 723 1	331.5
	C	173	38,751.5	224.0
	Total	407	93,602.8	230 0
Reissner membrane	More than	400	829.3	
Total membranous cochlea	A			294.2
	B			295.5
	C			292.2
	Mean			293.9

2 *Quantitative analysis of acid mucopolysaccharides*

It was found that the tectorial membrane contained AMPS, although the amount was very small (about 0 1 % per D W) Further enzymic analysis was not possible due to the small amount, however it was not necessary for the tectorial membrane to decompose DNA since it had no cell component (Iurato 1962) The O.D therefore indicated the existence of AMPS. The details are shown in Table 2.

Reissner's membrane also contained about 0 1 % of AMPS (Table 2) This was the data without decomposition of DNA. The possibility of contamination of DNA was about 0 05 % using this method (Table 3) so that the eval-

Table 2. *Analytical data of the tectorial membrane and Reissner s membrane*

Tissues	Series	D W (μg)	O.D (corrected) at 400 mμ	AMPS extracted (μg)	% D W
Tectorial	A	397.5	n.d	—	—
	B	657.9	.011	0.7	0.10
membrane	C	1,030.0	.018	1 1	0.10
	D	519 4	.012	0.7	0 13
Reissner membrane		829.3	.021	1.3	0.15

not detectable.

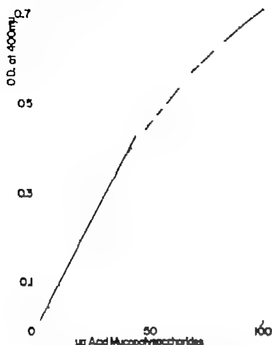


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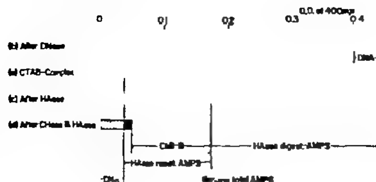


Fig 2 Enzymic analysis on acid mucopolysaccharides in the stria vascularis with the spiral ligament in series D

uation was for reference only It was, however suggested that the homogeneous ground substance (Iurato, 1967) in this membrane contained AMPS Enough stria vascularis with spiral ligament and basal membrane with the organ of Corti and limbus were collected to perform enzymic analysis. The materials of series A and B were used for preliminary experiments and for practice

Stepwise enzymic analysis on the stria vascularis with spiral ligament in series D was shown in Fig. 2. The precipitate extracted from them was dissolved in 4 0 ml of acetate buffer which was further treated according to the method described above. The O.D by CTAB-Li reagent before enzymic treatment [illegible] 432, which indicated a concentration of 47.5 μg per ml from the calibration curve (Fig. 1) Therefore, the total amount was 190 μg (47.5 × 4) The O.D after treatment with DNase was 0.395 which meant a concentration of 42 μg per ml and a total of 168 μg. The 168 μg was thus the genuine total of AMPS in this tissue. The difference of 22 μg, subtracting 168 from 190 μg, was the amount of contamination of DNA. The amounts of HAase digestive AMPS HAase resistant AMPS and ChS-B were obtained in the same manner (Table 3)

It was thus found from series C and D that the stria vascularis with spiral ligament contained 0 6% of AMPS dry weight, which consisted of 61% of HAase digestive AMPS and 39% of HAase resistant AMPS The basal membrane with the organ of Corti and limbus spiralis contained 0.5% of AMPS dry weight, which consisted of 74% of HAase digestive AMPS and 26% of HAase resistant AMPS. HAase resistant AMPS was almost all ChS-B in both parts. However there was a little other HAase resistant AMPS in the stria vascularis with spiral ligament. HAase digestive AMPS was almost likely to be HA as a result of the histochemical study (Saito, 1967) Very little difference between tissues was observed, which suggested similar structural characteristics. The details are shown in Table 3

DISCUSSION

The turbidimetric method originated by DiFerrante was modified to increase its sensitivity LiOH instead of NaOH (Hasegawa, 1969) made the O D at 400 mμ higher that is, from 0.500 to 0.585 at a concentration of 100 μg of ChS-A per ml. Moreover one ml of CTAB-Li reagent instead of 2 ml increased the sensitivity almost twice as much at a concentration of 50 μg per ml, namely from 0.250 to 0 450 The addition of 0.2 ml of NaCl (Hasegawa, 1969) increased the sensitivity especially with small samples. The turbidity of almost zero at 1 μg per ml was raised to 0 008 with the addition of NaCl, which made this concentration the minimum quantity detectable However the range of proportional linearity was 1 to 40 μg per ml.

The addition of NaCl (0.34 M Na-ion in total) was also considered to dissolve CTAB-complexes other than acidic polysaccharides.

Table 3. *Analytical data of the stria vascularis with the spiral ligament and the basal membrane with the organ of Corti and limbus spiralis*

Tissues	Stria vascularis with spiral ligament			Basal membrane with organ of Corti & limbus spiralis		
Series	C	D	Total	C	D	Total
Total D W (μg)	38 751.5	27 788.9	66,540.4	13,914 1	7,552.7	21 466.8
CTAB-complex (μg)	252	190	442	68	50	118
AMPS (μg)	240	168	408	60	46	106
(.D.W)	(0 6)	(0 6)	(0 6)	(0.4)	(0.6)	(0.5)
HAase digest.	132	118	250	40	38	78
(% AMPS)	(55)	(70)	(61)	(67)	(83)	(74)
HAase resist.	108	50[a]	158	20	8[b]	28
(AMPS)	(45)	(30)	(39)	(33)	(17)	(26)
DNA	12	22	34	8	4	12
(%D W)	(0.03)	(0 07)	(0 05)	(0.05)	(0 05)	(0 05)

ChS-B, 48; other 2.
ChS-B,8, other 0.

The solution extracted from the tectorial membrane always showed high turbidity after the addition of CTAB-Li reagent, but the addition of 0.2 ml of 2 M NaCl reduced it greatly For instance, the turbidity from the tectorial membrane in series C by CTAB-Li reagent was 0 085 which became 0 018 after the addition of NaCl. The concentration of sodium ion, 0.34 M, was lower than 0 4 M, which is the lowest concentration which dissolves the complex with AMPS (Schiller *et al* 1961) The high pH (12.5) caused by LiOH increases the charge density and reduces the specificity of the reaction for acidic polysaccharides (Scott, 1960) Therefore, this difference of turbidity was regarded as the turbidity of some neutral polysaccharides.

Iurato (1960) performed chemical analysis of the tectorial membrane and the spiral ligament by the method of Dische. He concluded that there were no AMPS in the tectorial membrane, and that there were 0.3% of them in the spiral ligament. In the present study we found 0 1% AMPS in the tectorial membrane, and 0 6% AMPS in the stria vascularis and spiral ligament. The sensitivity of the method employed may account for the difference between these results, however it is agreed that they are present in very small quantities.

Juhn & Niederwieser (1968) indicated that, by a thin layer chromatography AMPS in the stria vascularis with spiral ligament were similar to keratosulfate. On the other hand, the present enzymic analysis demonstrated two groups of AMPS HAase resistant (mainly ChS-B) and HAase digestive (mainly HA) AMPS in the same tissues. Purification of the material by dialysis may account for this difference because chromatography of AMPS is sometimes unstable, especially for salts.

It was found in the present study that the tectorial membrane contained 0 1% of AMPS This very small quantity may explain the discrepancies in the measurements of the tectorial membrane by other techniques, such as histochemistry and autoradiography However it is uncertain how such a small quantity could effect the polarization of the tectorial membrane.

Most of the mucopolysaccharidoses excrete an excess of ChS-B in urine (Schubert & Hamerman, 1968) The present study showed that the stria vascularis, spiral ligament and the basal membrane with the organ of Corti and limbus spiralis of the guinea pig contained ChS-B This is an interesting coincidence The excess or shortage of ChS-B may affect connective tissue which may lead to impaired hearing.

ACKNOWLEDGMENT

The authors are grateful to Associate Professor O Mizukoshi and Associate Professor E. Hasegawa for their suggestions and encouragement. Technical assistance of Mr R. Gore and Mr V McAuliffe is appreciated.

ZUSAMMENFASSUNG

Wir haben eine Trübheitsbestimmungsmethode, die auf der Bildung unlöslicher Komplexe zwischen sauren Mukopolysacchariden und Bromcetyltrimethylammoniak basiert, für die Mikroanalyse abgeändert. Wir benutzten diese Methode, zusammen mit einer Enzymanalyse um die Verteilung der sauren Mukopolysaccharide in der häutigen Schnecke des Meerschweinchens zu messen. Die tektorische, sowie die Reissnersche Membrane enthielt 0 1% saure Mukopolysaccharide pro Trockengewicht. Die Stria vascularis und das Ligamentum spirale enthielten ungefähr 0,6% ebenso die Basilarmembrane mit dem Cortischen Organ und dem Limbus spiralis. Als Referenz wurde das Durchschnittstrockengewicht verschiedener Teile der häutigen Schnecke an Hand einer Quarzschraubenwaage bestimmt. Die tektorische Membrane wog 4,6 µg pro Schnecke.

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THE EFFECT OF A BELOW THRESHOLD CONTINUOUS TONE ON THE THRESHOLD OF SUBJECTS WITH ABNORMAL ADAPTATION

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Six subjects with abnormal adaptation tracked their thresholds on Békésy audiometer for fixed frequency interrupted pure tone stimulus presented in conjunction with continuous tone of the same frequency at levels 20, 10 and 5 dB below that of the interrupted tone. The presence of the continuous tone below the threshold for the interrupted tone produced abnormal adaptation in four of the subjects, and narrowing of the excursion width of the tracings in three of the subjects. Effects of the below-threshold continuous tone were found in subjects with cochlear lesions, as well as in subjects with retrocochlear lesions. It is concluded that continuous stimulation does not have to be at threshold t produce abnormal adaptation in susceptible individuals.

Although the phenomenon of abnormal adaptation, as measured by Békésy audiometry and the various forms of the Tone Decay Test, has been found to be useful in the detection of auditory nerve lesions, very little research has been done into the stimulus conditions necessary for its occurrence It is not unlikely therefore, that there are important aspects of the phenomenon that are at present either unknown or are not measured by present techniques.

Recent research trends promise to rectify this situation. Jerger & Jerger (1966) using fixed frequency interrupted-tone Békésy audiometry on 5 subjects known to exhibit abnormal adaptation, varied the off time of the tone while holding the on-time constant, and found that there are critical off-times at which abnormal adaptation first begins to appear Dallos & Tillman (1966) investigated one subject known to have abnormal adaptation, again using fixed frequency interrupted-tone Békésy audiometry but varying both the on and off times of the stimulus. They found that, for the particular subject and the frequencies tested, stimulus duration did not exert any systematic influence on threshold, but that profound changes occurred in threshold as the off-time of the stimulus was decreased below 400 msec. They also found that under some conditions of frequency modulation of continuous pure tone stimuli, namely wide frequency deviation and slow repetition rate, it was possible to obtain from their subject stable thresholds that did not adapt abnormally

The purpose of the work to be reported here was to extend this research into the conditions of occurrence of abnormal adaptation by studying the effect of a below-threshold continuous tone on the interrupted-tone threshold, at the same frequency of subjects known to exhibit abnormal adaptation. There is no reason to believe that sound must be at or above threshold in order to affect the functioning of the auditory system. It is therefore reasonable to speculate that continuous stimulation does not have to be at threshold in order to produce abnormal adaptation in susceptible individuals

METHOD

Subjects

Six subjects were investigated, and all gave reliable results in Békésy audiometry Only one

Table 1 *Pertinent information on the subjects of the investigation*

Subject	Ear	Site of lesion	Aetiology	Type of Békésy audiogram
J A.	R	Cochlear	Cochlear otosclerosis	II
A. B	R	Retrocochlear	Acoustic neuroma	III
D J	[redacted]	Retrocochlear	Acoustic neuroma	II
L. M	R	Retrocochlear	Acoustic neuroma	IV
E. P	R	Cochlear	Skull injury	II
[redacted] W	R	Cochlea	Menière's disease	II

ear of each of the subjects was investigated This ear had a sensori-neural hearing loss, and exhibited abnormal adaptation (measured by the separation of the interrupted and continuous tracings in a sweep-frequency Békésy audiogram) at a number of frequencies. Three of the ears had hearing losses of cochlear origin, and three had losses of retro-cochlear origin. (Pertinent information is given in Table 1)

Apparatus

A block diagram of the apparatus used in the investigation is given in Fig. 1 The output from a Marconi variable frequency oscillator (Type TF 2100) was divided into two channels, one of which passed through a Marconi attenuator (Type TF 2162) the other through a Grason-Stadler electronic switch (Model 829-…), which was controlled by a Grason-Stadler interval timer (Model 471) The two channels were then mixed to form one channel and fed into the alternate input of a Grason-Stadler Békésy audiometer (Model E-800)

The attenuator channel provided the continuous component of the mixed signal, and the electronic switch channel provided the interrupted component of the signal. It was found that the attenuator and electronic switch introduced no phase difference between the two channels. The interval timer and switch were set so that the interrupted signal had a rise–fall time of 10 msec, an on-duration of 200 msec at maximum amplitude, and an off-duration of 250 msec, with the rise and fall times occurring during the 250 msec off-duration. The relative levels of the pulsed and continuous components of the mixed signal were monitored by means of an oscilloscope attached to the mixer output.

Procedure

The procedure carried out with each subject was as follows

(1) an ordinary sweep-frequency interrupted and continuous tone Békésy audiogram (100 Hz to 10 kHz attenuation rate 5 dB/sec) was obtained on the ear to be investigated

(2) the selector on the Békésy audiometer was then switched to the alternate input, and the channel from the mixer was introduced into the audiometer

(3) the oscillator was set at the frequency showing the greatest separation of the interrupted and continuous tone tracings in the Békésy audiogram

(4) the continuous channel was disconnected by means of a switch in the mixer and the subject tracked his threshold for the inter

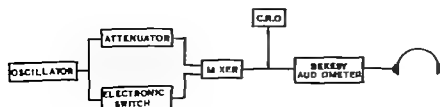


Fig. 1 Block diagram of the apparatus used in the investigation.

rupted tone alone for a duration of about 30 sec after an initial period of stabilisation

(5) the interrupted channel was disconnected, the continuous channel was switched in, and the subject tracked his threshold for the continuous tone, which had been set at the same intensity level as the interrupted tone, for about 30 sec

(6) both channels were then switched in, the continuous tone being introduced at an intensity level 20 dB below that of the interrupted tone, the level of which was kept constant by means of a continuously variable attenuator in the electronic switch the subject again tracked his threshold for this stimulus for about 30 sec

(7) the intensity of the continuous tone was increased to a level 10 dB below that of the interrupted tone, and the subject again tracked his threshold

(8) the intensity of the continuous tone was increased to a level 5 dB below that of the interrupted tone, and the subject tracked his threshold for this stimulus

(9) finally the subject tracked his threshold for the continuous tone alone again.

Appropriate instructions were given to the subjects for the various stimulus conditions. When the stimulus was interrupted, the subject was instructed to track his threshold for an interrupted tone. When the stimulus was continuous, the subject was advised to that effect, and instructed to track his threshold for a continuous tone. When the stimulus was a mixture of the interrupted and continuous signals, with the continuous component 10 or 20 dB below the level of the interrupted component, the subject was instructed to track his threshold for an interrupted tone, as the continuous component did not become observable However when the continuous component was 5 dB below the level of the interrupted component, the continuous component could occasionally be observed when the level of the interrupted component was allowed to rise above threshold, so the subject was advised that a continuous signal was present below the interrupted signal and might occasionally become observable but was instructed to ignore the continuous signal, and continue to track threshold for the interrupted signal.

The mean mid-points (MMP) of the Békésy tracings obtained from the subjects under the various conditions were calculated by averaging the same number of consecutive minimum and maximum points of the excursions (approximately 10 points each in the 30 sec test period), adding the average of the minimum points to that of the maximum points, and dividing by 2. The mean excursion widths

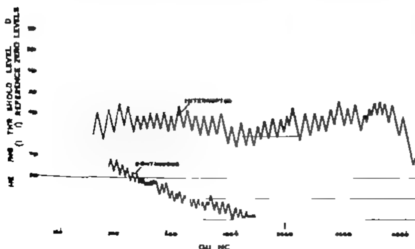


Fig 2 Sweep frequency interrupted and continuous tone Békésy audiogram obtained from the right ear I Subject A.B

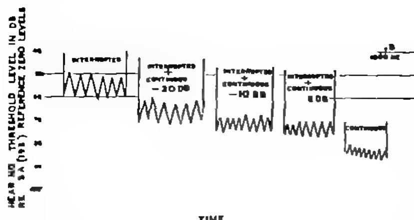


Fig 3 Fixed frequency traces obtained at 1000 Hz from the right ear of Subject A.B. under the specified experimental conditions.

(MEW) of the tracings were obtained by subtracting the average of the minimum points from the average of the maximum points.

RESULTS

Individual results for subject A.B are given in Figs. 2 and 3 Fig. 2 is the sweep frequency Békésy audiogram obtained from A.B s right ear Fig. 3 comprises fixed frequency traces obtained at 1000 Hz in A.B s right ear under the various experimental conditions It is clear that the introduction of the continuous 1000 Hz tone below threshold shifts the threshold wnwards and tends to narrow the excursion th of the trace. It was noted that the sepa ation of the interrupted and continuous tone tracings obtained in the sweep frequency Békésy audiogram was greater than that obtained under the fixed frequency presentation. This was assumed to be due to a cumulative effect resulting from the sweep frequency mode of presentation. The downward trend of the continuous tone fixed frequency tracing supports *this hypothesis.*

Table 2 gives the mean mid-points and mean excursion widths of the fixed frequency tracings obtained from the subjects under the various experimental conditions. The MMPs are given in dB re ASA (1951) Reference Zero Levels. The MEWs are expressed in dB In the case of subjects D.J and G W the changes in threshold (MMP) and MEW as the below threshold continuous tone was introduced and its level increased were very small and may well have been due to retest variability It is quite possible, then, that, in these subjects, the introduction of below-threshold continuous

Table 2. *Mean mid-points (MMPs) and mean excursion widths (MEWs) of the fixed frequency tracings obtained from the subjects under the various experimental conditions*

MMPs are given in dB re ASA (1951) Reference Zero Levels. MEWs are in dB

Subject	J A.		A. B.		D J		L. M.		E. P		G W	
Test frequency	4,000 Hz		1,000 Hz		4,000 Hz		5,000 Hz		2,000 Hz		7,000 Hz	
Tracing characteristic	MMP	MEW	MMP	MEW	MMP	MEW	MMP	MEW	MMP	MEW	MMP	MEW
Interrupted	69.2	7.6	55.5	10.7	55.5	9.9	40.0	19 6	35.5	10.2	52.5	10 3
Interrupted + continuous (−20 dB)	67 1	6.8	67 8	12.0	54.5	11 7	45 7	20.5	35 1	8.0	53.5	9 2
Interrupted + continuous (−10 dB)	71 4	6.6	71.3	8.3	55.9	10.3	58.4	15.5	36.7	7.5	53.9	7 5
Interrupted + continuous (−5 dB)	75 7	6.3	74.3	7.8	57.8	8.4	79 7	13 4	40.8	6.9	54 6	8.0
Continuous	77 7	4.2	84.3	5 4	62.5	5 7	87.3	13.1	42.5	5.8	60.0	7 0

stimulation had no effect. However in the case of subjects J.A., A.B L.M. and E.P., the gradual introduction of the below-threshold continuous tone produced a significant drop in threshold, and, in the case of subjects A.B., C.M., and E.P., a significant narrowing of the MEW

DISCUSSION

The results indicate that below-threshold continuous stimulation can influence the auditory functioning of subjects with abnormal adaptation. On the present rather scant evidence, it would seem that the drop in threshold and narrowing of tracing excursion width produced by below-threshold continuous stimulation can occur in abnormally adapting subjects with cochlear lesions, as well as in those with retrocochlear lesions. If this is the case, then the occurrence of these effects cannot provide a means of distinguishing retrocochlear from cochlear lesions. However further investigation would be necessary on this question, and to discover whether there are any characteristics of the effects of below-threshold continuous stimulation that are distinctive to retrocochlear lesions.

The present evidence also suggests that the effects of below-threshold continuous stimulation can occur in subjects with small amounts of abnormal adaptation, as well as in those with large amounts. Again, further investigation would be needed *to establish whether this* is so or not, and to discover whether there is any relationship between the effects of below-threshold continuous stimulation and degree of abnormal adaptation.

Finally it seems likely that other forms of below-threshold continuous stimulation, such as wide and narrow bands of white noise, would have similar effects on abnormally adapting subjects.

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ZUSAMMENFASSUNG

Békésy Audiogramme für einen frequenzkonstanten, pulsierenden Reinton plus einen stetigen Ton der selben Frequenz mit Intensitäten 20, 10 und 5 dB unter der Intensität des pulsierenden Tones wurden von sechs Patienten mit anomaler Adaptation aufgezeichnet. Beim dem stetigen, unterschwelligen Ton fand sich anomale Adaptation in vier Patienten, und Reduktion der Kurvenausschläge in drei Patienten. Diese Reaktionen wurden in Patienten mit cochlearen Läsionen, sowohl auch in Patienten mit retrocochlearen Läsionen, beobachtet.

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STAPEDIOLYSIS OR STAPEDECTOMY

A Comparison based on a 3-years Follow-up Material

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The material studied consisted of 176 persons oper ated on for otosclerosis, the majority of whom were treated by stapediolysis, using the Fowler–Holmgren method, and the rest by stapedectomy using Schuknecht's method. Hearing tests were done both one week and three years after operation and showed that an improvement occurred in about 85% of the patients in each of the two groups. The relatively good results in the stapediolysis cases were ascribed to the meticulous care with which the operation was carried out. Where the intended improvement in hear ing was not achieved—e.g., where fracture of the posterior crus occurred—stapedectomy was then per formed in the same operation session. Attempts to discover why 1 -14% of the cases showed a subse quent deterioration in hearing or no improvement have, in spite of careful analysis, proved fruitless. reason would appear to be of temporary or al nature.

the extensive literature concerning operative therapy in cases of otosclerosis two factors are predominant—namely surgical technique and case results. From the original mobilisation technique described by Rosen (1953 1955) and subsequent developments in stapediolysis suggested by Fowler (1956) and described by Holmgren (1957) various methods of stapedec tomy have gradually been brought into use. In recent years a number of articles have been published describing increasingly impressive results in otosclerosis operations (Goodhill, 1956 Bellucci, *1959 1961* Kos *et al. 1960* Cody *et al.* 1965 Kristensen, 1966). Attempts have also been made to analyse the reasons why in spite of everything, unsuccessful cases still occur Not least significant are those inexplicable failures that lead to sensory-neural loss.

On the basis of a careful follow-up of cases from the years 1963–1965 we propose to show the favourable results of a comparatively simple operation such as stapediolysis, using Fowler's method, which Holmgren reported as far back as 1957 We shall also try to analyse the unsuccessful cases to discover why no improvement took place.

MATERIAL AND METHOD

The material consists of 176 cases with a diagnosis of otosclerosis, operated on during the period 1963–1965 There were 104 women and 72 men, of whom 68% were between 40–60 years old at the time of operation. Five patients were under 20 years old Thirty per cent of the patients had a definite hereditary background.

The follow-up examination was planned and carried out at intervals of 1 week, 1 month, 6 months and 3 years after operation. All 176 patients were examined within about 1 week after operation, while 171 could be examined up till 3 years had elapsed. In the 5 cases where the 3-year examination could *not* be made the reason was geographical. In all cases the previous postoperative examination had shown a very satisfactory improvement.

Table 1 classifies the material by Sham-

Table 1 *The material classified according to Shambaugh's prognosis groups*

Group	Number of patients	Per cent
A	13	7
B	65	37
C	54	31
D	44	25
Total	176	100

baugh's method, where *A* means bone-conduction 0–15 dB *B* bone-conduction 16–25 *C* bone-conduction 26–35 and *D* bone-conduction above 35 dB As will be seen, groups *C* and *D* constitute 56% of our material. The reason for greater severity on initial examination in many cases seems to be geographical. Where the patients have to travel long distances they tend to visit the physician relatively late, which results in the average age being comparatively high.

Table 2 shows the operations undertaken, the two predominant types being stapediolysis and stapedectomy using Schuknecht's method. Stapediolysis was performed by the Fowler-Holmgren method which consists mainly of dividing the plate and cutting through the anterior crus, which isolates the otosclerotic focus while freeing the anterior part of the plate. Stapedectomy using Schuknecht's method, was performed with a prosthesis consisting of a steel wire to which fat from the ear lobe was attached.

Table 2. *Cases classified according to type of operation*

Operation	Group A	B	C	D	Number of patients	Per cent
Mobilization	1	1	1	2	5	3
Stapediolysis	10	29	25	16	80	44
Stapedectomy (Schuknecht)		26	16	15	57	33
Stapedectomy (Shea)		5	6	6	17	10
Partial stapedectomy (Portmann etc.)	2	4	6	5	17	10
Total	13	[illegible]	54	44	176	100

Follow-up examinations of the 176 cases were carried out at the ear clinic's audiological department by means of pure tone audiogram, and, in several cases, speech audiogram. The latter however was not used as a rule. The previously-mentioned intervals between the examinations were maintained, in spite of the long distances to be travelled. Several patients were unable to follow this schedule but for all of them it was possible to carry out the 1 week, 10-day and 6-month examinations. The 3-year examination, as stated, was missed in only 5 cases. In special circumstances—for example, where hearing deteriorated—more frequent examinations were made.

Seven cases, in which stapediolysis was carried out without any resultant improvement in hearing during the following 3-year period, were operated on again. The second operation, which fell outside the time-limit of our examination, is not included in this study

RESULTS

General

The postoperative improvement in hearing was analysed at 7–10 days and 3 years after operation (Fig. 1)

Tables 3 and 4 show that one week after operation 24 cases (i.e., 14%) had deteriorated or failed to improve. Three years after operation the corresponding figures were 21 cases and 12% Nineteen and 16 of the cases belonged to prognosis groups *C* and *D* respec

Table 3 *Hearing status 1 week after operation*

Hearing gain in dB	Group A	B	C	D	Number of patients	Per cent
41–50		1			1	1
31–40	3	5	5	2	15	9
21–30	4	19	14	9	46	26
11–20	3	28	14	13	58	32
5–10	2	8	11	11	32	18
No gain	1	2	8	8	19	11
Impairment		2	2	1	5	3
Total	13	[illegible]	54	44	176	

Table 4 *Hearing status 3 years after operation*

Hearing gain in dB	Group A	B	C	D	Number of patients	Per cent
41 50	1	2	2		5	3
31–40	1	12	8	10	31	18
21 30	3	20	13	4	40	24
11 20	4	[illegible]	21	[illegible]	66	39
5–10	2	2	2	2	8	4
No gain	1	3	5	8	17	10
Impairment		1	2	1	4	2
Total	12	[illegible]	53	[illegible]	171	100

tively a definite over representation which agrees well with the prognosis.

It is also of very great interest to study the air–bone gap in all the 176 and 171 patients mentioned. A run-through of the material shows that thus drops from 34 dB before operation to 17 dB one week after and 12 dB 3 years after the operation (Fig. 2)

Comparison between stapediolysis operation using Fowler s method and stapedectomy using Schuknecht's method

These two operations, which together account for 137 of the 176 operations (77%) were subjected to a comparative analysis (Tables 5 and 6) It will be seen that stapediolysis, using



Fig 1 Hearing status in the total material 1 week and 3 years after operation.

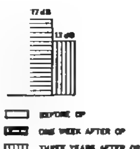


Fig 2 The air–bone gap in 171 patients before and after operation.

Fowler s method, yields surprisingly good results. No fewer than 65 of the 78 cases (84%) showed an improvement of more than 10 dB after 3 years. None of the operations carried out by Fowler's method led to a deterioration in hearing. The operation using Schuknecht's method gave almost identical results, though two of them showed deterioration after 3 years.

A study of the air–bone gap in the 3-year examination for both groups likewise showed almost complete agreement (Fig. 3)

Analysis of cases that showed neither improvement nor deterioration

As can be seen in Table 3 19 patients showed no improvement in hearing 1 week after the

Table 5 *Hearing status following stapediolysis, using Fowler s method*

Hearing gain in dB	One week after operation: Number of patients	Per cent	Three years after operation: Number of patients	Per cent
41 50			2	3
31–40	10	12	8	10
[illegible] 30	21	27	21	27
11 20	29	36	34	44
5–10	15	19	4	5
No gain	5	6	9	11
Impairment				
Total	80	100	78	100

Table 6. *Hearing status following stapedectomy using Schuknecht's method*

Hearing gain in dB	One week after operation		Three years after operation	
	Number of patients	Per cent	Number of patients	Per cent
41–50	1	2	3	5
31–40	3	5	10	18
21–30	16	28	13	4
11–20	20	35	22	40
5–10	9	16	2	4
No gain	5	9	3	5
Impairment	3	5	2	4
Total	57	100	55	100

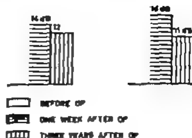


Fig. 3. Comparison in air-bone gap between 78 patients with stapediolysis and 55 patients with stapedectomy.

operation. In these cases mobilisation of stapes was carried out on 3 patients. Five patients were operated on using Fowler's method and 5 patients using Schuknecht's method. Three patients were operated on using Shea's method and partial stapedectomy using Portmann's method was performed on 3 patients. The Table also shows that it was mainly patients with advanced otosclerosis in groups *C* and *D* who were affected.

Table 3 also shows that a deterioration in hearing was found in 5 patients 1 week after operation. Four of these had the Schuknecht operation and one the Shea-operation. It should be noted that no patient operated on using Fowler's method showed deterioration in hearing. In 2 patients the deterioration is explicable. One of them, according to the operation record, in addition to the otosclerotic focus, had a classical tympanosclerosis with many adhesions between the crura of the stapes, the facial nerve and the promontory. Here the Schuknecht-operation brought no improvement. The other patient had a Shea-operation. Acute otitis developed 3 days after operation. In spite of intensive treatment, deterioration in hearing occurred, but no total deafness. An examination of 17 patients 3 years after operation showed no improvement in hearing. Of these patients 2 had stapes mobilisation, 9 stapediolysis, while 3 patients had a Schuknecht-operation. Two patients had a Shea-operation and 1 a stapedectomy according to Portmann.

Four patients were found to have deterioration in hearing after 3 years. Of these, 2 were operated on using Schuknecht's method, 1 using Shea's method vein-graft and 1 Portmann's. Two patients had a serious deterioration in hearing. No deterioration after 3 years was found in patients with stapediolysis, although this is the largest group.

Operation records for patients who showed no improvement and for those with deterioration in hearing after 3 years give no clue to the causes of failure, but in some cases it is mentioned that the operations were very difficult technically because of different anatomical variants.

DISCUSSION

There are two main questions of interest in the foregoing material which, admittedly is modest in comparison with many previous studies but which, on the other hand, can boast a considerably higher follow-up percentage. The results obtained as regards hearing are not without interest, but they are excelled by other data. The reason for the relatively results from our material is ch

percentage of cases with unfavourable prognosis, using Shambaugh s scale.

An interesting fact is that it is possible to compare data from cases in which stapediolysis was performed using Fowler s, or rather Holmgren s method (so-called PENN-operation. Holmgren, 1957) Most of these operations are carried out in such a way that the plate is divided with a pointed needle, after which the anterior arm is sawn off with a diamond disk. Thus the isolated anterior part of the otosclerotic focus is excised in the simplest possible manner Sometimes, this has not succeeded, but the gap between the remaining part of the plate and the surrounding area has become large enough to permit a satisfactory degree of mobility For the operation to yield good results one must be absolutely sure that the posterior crus has a completely unbroken connection with the remaining part of the plate, so that the posterior crus and the plate function like a *pelott* in the hole. We have reason to believe that the poor results of stapediolysis are due to breaking of the posterior crus just behind the point of attachment to the plate. Some of these can be ascertained only by a very close inspection of the area. A sufficiently wide approach is therefore often a necessary condition for success. If during the operation it appears that continuity between the posterior crus and the plate is broken, the operation

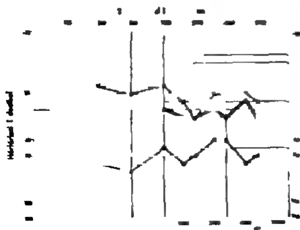


Fig. 4 Typical acute hearing gain following stapediolysis. (*Double curve*)

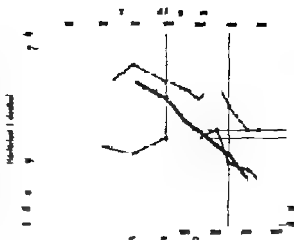


Fig 5 Typical acute hearing gain following stapedectomy using Schuknecht's method. (*Double curve.*)

should be continued by means of stapedectomy using Schuknecht s method. By employing the 1 2-test, introduced by Holmgren, it is very easy to discover whether one has succeeded or not. If stapediolysis is performed as described above good initial results may be expected, as well as very good longterm results, as shown in the Tables.

The postoperative course in stapediolysis cases is generally quite free from complications, not least as regards dizziness, which is almost entirely absent in these cases. Moreover after the stapediolysis operation no deterioration of neurogenic type occurred. In this connection we consider it important to point out the well-known immediate hearing injury of neuro-sensory type in the upper register that results from stapedectomy This does not occur with stapediolysis (Figs. 4 and 5) In the stapedectomy cases the patient s condition gradually returns to normal, but probably not always. In 7 cases where stapediolysis brought about an improvement, deterioration subsequently occurred stapedectomy using Schuknecht s method, was therefore performed in all cases and resulted in a remarkable improvement in hearing.

We are thus very much inclined, in spite of the wide and generally favourable experience with stapedectomy throughout the world, to

emphasize the positive aspects of the conservative operation that stapediolysis really is It is thus possible for many patients to achieve perfect hearing without the introduction of a foreign body into the middle-ear

The other problem that interested us—and it is naturally much more difficult to solve—is why no improvement occurs, or a deterioration takes place in cases operated on in a perfectly satisfactory manner In spite of careful analysis of operative notes and of the postoperative course, and the possibility of obtaining all imaginable ancillary data on this small number of cases, it has not proved possible to answer this question. It seems likely that chance variations in operative technique may be responsible. Obviously the more the technique is developed, the greater the likelihood of a good result.

ZUSAMMENFASSUNG

Ein Patientengut, bestehend aus 176 wegen Otosklerose Operierter wurde analysiert. Der grösste Teil operiert teils mit Stapediolysis nach Fowler Holmgren und teils mit Stapedektomie nach Schuknecht. Das Gehör wurde eine Woche als auch drei Jahre nach der Operation geprüft, wobei Gehörverbesserungen von ungefähr 85% registriert wurden. Dieses gilt für Stapediolysis und Stapedektomie. Das verhält nismässig gute Resultat bei der Stapediolysis wird der sorgfältigen Kontrolle während der Operation zugeschrieben. In den Fällen in denen das gew. nschte Gehörresultat nicht erreicht wurde, z.B bei Auftreten eines Schenkelbruchs, wurde die Stapedektomie in gleicher Sitzung vorgenommen.

Die Ursache der Gehörverschlechterung oder nicht eingetretenen Gehörverbesserung bei 12 14% konnten trotz sorgfältiger Analyse der Operation und des postoperativen Verlaufs nicht festgestellt werden. Die Gründe dürften in mehr oder weniger zufälligen Faktoren zu suchen sein.

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LIPIDOSIS OF THE BASILAR MEMBRANE

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Lipidosis of the basilar membrane was described as a type of presbyacusis. The concept was based on observations of 24 ears from 20 patients, whose ages ranged from 71 to 95 years. Several staining procedures were employed to the basilar membrane Six ears from younger patients were used as a control Twelve ears from 9 patients showed lipids deposit on the basilar membrane along the filamentous structure of the pars pectinata. The deposit found in the basal turn ranged from the basal end up to 10 mm area. There was more of it nearer the basal end and less apicalwards. The organ of Corti in that area was either present or missing. The lipids were composed mainly of neutral fat and a small amount of cholesterol. Acoustic significance of the lipidosis was discussed.

When the sound sets the labyrinthine fluid in otion, the basilar membrane contributes to transmit vibrations to the organ of Corti. The stimulation of the hair cells is effected by inter action between the fluid and the basilar mem brane, propagating the sound along the cochlea. There is an orderly spatial distribution of frequency along the cochlear duct. When transmitting high tones, the travelling wave reaches maximum amplitude in the basal region and low tones in the apical region

Any mechanical or chemical changes which impede the vibration of the basilar membrane may cause a kind of conductive hearing im pairment. However in this regard, little attention has been paid to the basilar membrane, since the membrane scarcely shows pathology under the light microscope Even the intense noise and ototoxic drugs hardly produce noticeable morphological changes in the basilar membrane.

The purpose of the present paper is to de scribe morphological features of the basilar membrane of elderly patients, which cannot be seen in conventional celloidin specimens. The author would like to propose the name Lipidosis of the Basilar Membrane" as one of the histopathological types of presbyacusis

MATERIAL AND METHODS

Twenty-four human temporal bones from 20 patients were submitted to this study The age of the patients ranged from 71 to 95 years. The postmortem hours at the time of the fixation were from 1 to 11 Duration of fixation in 10% formalin was from 2 to 7 months.

The temporal bone was decalcified with 5% solution of trichloracetic acid. The decalcified bone was then neutralized with sodium sulfate and washed in running water The bone was trimmed and the membranous labyrinth was exposed. For the convenience of further staining procedures, the cochlea was sectioned into two or three pieces.

During the staining procedures, the basilar membrane was kept attached to the osseous spiral lamina. In some cases ultrasonic shaking was applied to the tissues in order to disintegrate and detach the organ of Corti from the basilar membrane, prior to or after staining.

The following staining procedures were employed.

1 *Osmium tetroxide-α-naphthylamine (OTAN) method (Adams 1965 Elleder & Lojda, 1968)*

The tissue was incubated overnight in a solution of a mixture of 1% solution of osmium tetroxide (3 parts) and 1% solution of potassium chlorate (1 part) at room temperature. The tissue was then transferred to a saturated solution of α-naphthylamine, in which the incubation was carried out for 20 min at 37 C.

2. *Sudan III staining*

0.2 g of sudan III was dissolved in 100 ml of 70% alcohol. The tissue was stained for 30 min at room temperature. Afterward, it was differentiated in 50% alcohol and then brought into distilled water

3 *Nile blue sulfate method*

The tissue was stained in 1% solution of nile blue sulfate for 5 min at 60 C. The tissue was differentiated at 60 C in 1% solution of acetic acid for 1 min. The tissue was washed in tap water

4 *PAS reaction*

The tissue was transferred to 1% periodic acid reagent and kept there for 5 min. Then, it was washed in tap water for 3 min. The tissue was placed in Schiff's reagent. It was washed in tap water

5 *Smith-Dietrich reaction (Dietrich 1910 Smith & Mair 1911)*

The tissue was chromated in 5% solution of potassium bichromate for 24 hours at 37 C After washing in distilled water it was stained by Kultschitzky's hematoxylin solution for 5 hours at 37 C. The tissue was differentiated in Weigert's borax-potassium ferricyanide solution for 10 min. Phospholipids was stained blue black.

6. In order to demonstrate presence of fluorescence and birefringence, the unstained specimen was observed in ultraviolet light and in polarized light.

Stained tissue was washed in distilled water and mounted as a flat specimen in polyvinyl pyrrolidone medium.

FINDINGS

The basilar membrane showed its filamentous structure of the pars pectinata when its surface preparation was observed by optical sectioning. There were deposits of lipids along the filamentous structure of the pars pectinata in the lower basal turn (Fig. 1 B) Nine ears from 12 patients out of 24 ears from 20 showed lipids deposit in the basilar membrane Six cochleas from younger patients (1 24 37 years) did not show any deposit at all

It was stained from brown to black by OTAN method. In specimens from which the organ of Corti was detached by ultrasonic shaking, the lipids deposit was more clearly observed It existed most prominently in the basal turn from the vicinity of the basal end to 5–10 mm area, more heavily near the basal end and less apicalwards (Fig. 2 A and 2 B Table 2)

The apical and the middle turns failed to show deposit in the basilar membrane.

The results of varying procedures were summarized in Table 1 The deposit was soluble in ether-alcohol. It was sudanophilic, stained pink by nile blue sulfate and partly showed birefringence under cross-nicols. The deposit was composed mainly of neutral fat and small amounts of cholesterol. No reactions by PAS and Smith-Dietrich method indicated the absence of glucolipid and phospholipid respectively As the deposit did not fluoresce the presence of lipofuscin was not likely

Irrespective of the organ of Corti, some specimens had deposit, while others did not have any

DISCUSSION

The basilar membrane is composed of ground substance, filaments and of a few connective



Fig 1 A. Low power view of the osseous spiral lamina with the basilar membrane. The nerve fibers are orderly arranged. The inset is a part of the basilar membrane, the area which corresponds to Fig. 1 B OTAN stain, 9

tissue cells. The inner part of the membrane is termed the pars tecta. It is formed with radially arranged filaments, which are not grouped in bundles and lies side by side in scanty ground substance Beneath the filaments, there is some cottony ground substance without filaments. The outer part of the membrane is thicker and striated and is termed the pars pectinata. It is formed with compactly arranged grouped filaments, all of which run radially The space between the filaments is filled with a scanty ground substance. The fibers are separated from each other by an abundant cottony ground substance and are arranged in two strata (Iurato 1962, 1967).

The basilar membrane is broad in the apical turn, attaining the maximum width of about *500 μ* before the apical end, and narrow in the basal turn, measuring narrowest 80 μ at the end near the windows (Wever 1938)

Mayer (1920) stated that aged persons of over 60 years showed thickening of the basilar *membrane Calcerous deposit and occasional* ossification was also observed in the thickened

Fig. 1 B. Lipids deposit on the basilar membrane after ultrasonic shaking. It is along the filamentous structure of pars pectinata. The vague line running transversely down in the picture is vas spirale. OTAN stain, 140.

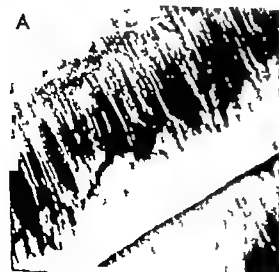


Fig. 2 A. 86 years female. Marked lipids deposit on the basilar membrane 1.3 mm area.

membrane. The rigidity of the basilar membrane was what he thought to be the anatomical substrate of presbyacusis. According to Mayer diseases such as atheromatose and marasmus produce degenerative changes of the labyrinth as well as loss of nerve fibers. Crowe *et al.* (1934) also reported similar findings at the very basal end of the basilar membrane in specimens from elderly patients, consisting of hyalinization and calcification. Glorig & Davis (1961) were of the opinion that there exists inner ear conductive presbyacusis in elderly patients with no recruitment. The patients showed gradual high tone hearing loss. They believe that the most plausible interpretation was a physical change in certain tissues of the cochlear partition. Schuknecht (196 postulated that hearing loss with deg

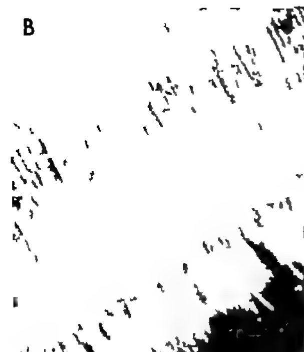


Fig 2 B. Deposit is less in 9 mm area. OTAN stain, 120.

audiometric curve is possibly caused by stif fening of the basilar membrane. He termed this type of audiogram in aged people as mechanical presbyacusis.

The present findings demonstrated the lipids posit as being mainly neutral fat and choles- l As formalin solution is not the best fixaé for lipids, there is a possibility that certain constituents were dissolved during the fixation (Weil, 1929 Halliday 1939)

The basilar membrane is a tissue usually de void of discernible lipids (Schätzle & Western hagen, 1967) The formation of fat in tissue other than adipose tissue is called aberrant lipogenesis, which is considered to be relevant to fatty degeneration and atheromatosis (Cogan & Kuwabara, 1960)

It is not clear how the lipids are deposited in the basilar membrane In this connection, the value of cholesterol content in the serum of the present patients had no correlation with the presence of the lipids deposit on the basilar membrane. As the deposit occurred in the lower basal turn, the degenerative process of

Table 1 *Histochemical property of the lipids*

Staining procedures	Results
Osmium	+
Sudan III	+
Nile Blue Sulfate	+ (pink)
PAS	–
Smith-Dietrich's Reaction	–
Birefringence	present (partly)
Autofluorescence	not present

the organ of Corti might be responsible for the formation of fat in the basilar membrane. At times, however the lipidosis was found in the basal turn where the organ of Corti was present. The term of lipidosis of the basilar membrane seems to be appropriate for pathol ogy

Next, the acoustical significance of the present findings should be considered. Impedance of transmission of vibrations to the organ of Corti is roughly expressed by the following equation (Portmann & Portmann, 1961)

$$I=\sqrt{r^2+\left(m\ f+\frac{s}{f}\right)^2}$$

I impedance *r* friction *m* mass *f* frequency of the acoustic vibration *s* stiffness.

Predominance of lipids deposit in the basal

Table 2. *Range of lipids deposit in the basilar membrane*

Lipidosis is found in 12 ears out of 24 (9 patients/20 patients). Deposit is observed in the membrane from the basal end to 5 10 mm area

Age	Sex	Distance from the basal end, mm
79	♂	5
86	♀	5
86	♀	6
73	♂	6.5
79	♂	7
84	♀	7
84	♀	7
86	♂	7
71	♂	8.5
86	♀	9
95	♀	9
75	♀	10

turn increases the value of *m f s* and *r* which in turn produces an audiometric curve of high tone loss. However it is hard to decide whether the lipids deposit is only the cause of hearing loss, because the lower basal turn frequently shows marked degeneration or loss of sensorineural elements.

ACKNOWLEDGMENT

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ZUSAMMENFASSUNG

Es wurden über die Lipidosis der Basilarmembran, die als ein Typ der Presbyacusis betrachtet wird, bei 20 Patienten (24 Ohren) 71–95 Lebensjahre beobachtet. An der Basilarmembran wurden verschiedene Farbungen vorgenommen. Als Kontrolle dienten 6 Ohren von jungen Leuten. Bei 9 Fällen von Alten, an 12 Ohren, zeigte sich an der Pars pectinata der Basilarmembran die Ablagerung von Lipid, die an der fadenformigen Struktur der Pars pectinata beobachtet wurde. Diese Ablagerung kam in der Basalwindung im 10 mm Teil von dem Basalende zum Vorschein. Diese Lipidosis war in der Nähe des Basalendes deutlicher weniger in der apikalen Richtung. Das Cortische Organ in diesem Teil war bald vorhanden, bald nicht vorhanden. Die Lipidablagerung bestand hauptsächlich aus dem Neutralfett und wenigem Cholesterin. Es wurde weiter uber die akustische Bedeutung bei Lipidablagerung erwähnt.

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HEREDITY IN A STRAIN OF THE WALTZING GUINEA PIG

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The genetic characteristics of a strain of waltzing guinea pigs with hereditary progressive deafness has been studied. The mode of inheritance was found to be due to a monohybrid dominant Mendelian autosomal gene with recessive lethal effect and with full expressivity and penetrance.

The scientific interest in the waltzing phenomenon in rodents is a rather recent one, al though there are ancient descriptions of the Japanese waltzing mouse, bred since at least 80 B.C. (Keeler & Fuji, cit. Nachtsheim, 1938 Grüneberg, 1947) An increasing number of publications have appeared since the end of the 19th century and geneticists have been able to define more than 17 mutants in dif rent rodents with a waltzing or waltzing-like haviour with or without cochlear defects (e g. Dice, 1935 Nachtsheim, 1938 Watson, 1939 Cogan, 1943 Grüneberg, 1943 and 1947 Altmann, 1950 Deol 1954 and 1965 *a b* Hadorn, 1955 Kocher 1960 Altman & Dittmer 1962)

The waltzing guinea pig was first described by Ibsen & Risty in 1929 as follows. "Two related individuals with a tendency to whirl, or waltz, similar to that known in the Japanese waltzing mice, arouse in the guinea pig stock of the senior author Breeding tests indicated that this mutation is probably as in mice, a simple Mendelian recessive." Ibsen (1932) and Lurie (1939 1940 and 1941) later confirmed these observations.

During an endeavour to elucidate the morphology physiology and possibly also the biochemistry involved in hereditary progressive deafness in a strain of waltzing guinea pigs it has been necessary to define exactly the mode of inheritance of this strain. It soon became evident that in contrast to earlier works the waltzing character in the present strain had a dominant rather than a recessive mode of inheritance Therefore, the following symbols have been used in this work: $W/+$ = waltzer $+/+$ = normal. The constitution W/W has not been seen and is, as will be shown, lethal.

MATERIAL AND METHODS

The present strain originates from six waltzing guinea pigs obtained from the National Institutes of Health in 1961 From then until 1966 they were bred at random with normal guinea pigs from an outbred Swedish strain. When the present investigation started the stock consisted of 51 animals, of which 16 were waltzers. No further animals except the original 51 and their progeny were used in the present study

All animals were identified by sex, fur colour body weight and vestibular function The vestibular function was tested by rotation, where the waltzers showed no nystagmus, as well as by tilting, where no counter-rolling could be seen in the waltzing animals. At birth all animals had elicitable Preyer reflex, all the waltzers, however lost this characteristic within 4–6 weeks of age.

Table 1

Parents' genotype		Number of offspring with different genotypes					
	Dead	W/+ Males	W/+ Females	+/+ Males	+/+ Females	Total
+/+ +/+	12			39	57	108
W/+ +/+	31	120	116	118	135	520
W/+ W/+	133	90	95	51	55	424
Total	176	210	211	208	247	1052

The mature guinea pigs were grouped for breeding in cages containing one male and two females in each. The animals were counted daily and the body weight of the females was recorded at suitable intervals. Body weight turned out to be a very efficient method for detecting pregnant and non-fertile animals. The pregnant females showed a steep increase in weight and many could double their body weight in two months. Stillborn animals were counted and discarded. Newborn animals were registered according to sex, fur colour weight and vestibular function, i.e waltzer or not.

It was almost always possible to establish who was the mother of the litter since only two adult and repeatedly weighed females were kept in each cage The father of the litter was always known. To prevent uncontrolled breeding care was taken to separate the litter from their parents at about one month of age or when the young had reached about 200 g in body weight. The offspring were then divided according to sex and kept in separate cages during adolescence Nevertheless, matings between the first and second generations occurred occasionally

The chromosomes were examined in one male waltzer

RESULTS

Different crosses were performed and the results are given in Table 1 The offspring amounted to 1060 individuals, 884 (83 4%) living and 176 (16 6%) stillborn. The 176 perinatally dead ones were apparently normally developed without obvious malformations, though many had been partially eaten up Sometimes only part of a limb was found, and it seems not unlikely that there had been more than 176 stillborns. It was impossible to find out whether they had been born alive or not. Only a few were found but feebly alive immediately *post partum* but in their moribund state they soon expired. Judging from the parents behaviour they were killing their moribund offspring.

Of the 884 living animals 456 appeared normal while 428 were waltzers there were 421 males and 463 females. The parents were known for 1052 newborns but remained unknown for eight from two pregnancies, these have been excluded from Table 1 The 1052 newborns have been divided into three groups according to the presumptive genetic constitution of their parents. Group one consisted of the offspring where both parents were normal (+/+ +/+) group two of the offspring where one of the parents was waltzer and the other normal (W/+ +/+) and group three of the offspring where both parents were waltzers (W/+ W/+)

The litter size was studied in the different crosses and the results are given in Table 2.

By caesarian section 36 fetuses were ob-

Table 2

Parents genotype		Offspring per litter Mean	s.D.	[illegible]	[illegible]	Litters
/	/	2.70	1.02	2.7	1.6	40
W/	+/+	2.88	1.20	2.8	1.9	181
W/	W/	58	1.27	2.4	1 4	164

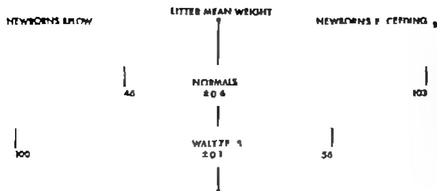


Diagram 1 Weight distribution of normal and waltzing littermates in mixed litters. The numbers signify those newborns exceeding or below litter mean weight. Six normals and one waltzer had a birth weight = litter mean weight. Further explanations: see text.

tained from 13 heterozygous females mated to heterozygous males. One of the fetuses was gravely undeveloped and dead while 35 were of normal appearance and alive

Each newborn was weighed as early as possible Litters containing both normal and waltzing littermates were studied. The mean weight of each litter was calculated, and the deviation of the birth weight of each individual within the litter from this mean was expressed as + (= more than the mean litter weight) or − (= less than the mean litter weight) In this way 312 newborns were examined, 155 normals (74 males and 78 females), and 157 walzers (79 males and 78 females) The distribution as given in diagram 1 is obtained.

Investigation of the chromosomes showed the normal number i.e $2n = 64$ There was a normal bivalent number in the meios, normal XY-bivalent and no structural chromosomal aberration (Fig. 1) (By courtesy of Assistant Professor J Lindsten.)

DISCUSSION

The results of different crosses as given in Table 1 shows conclusively that the mode of inheritance in the present strain of waltzing guinea pigs is a simple dominant Mendelian.

Normals mated to normals gave only normal offspring. The perinatal mortality was surprisingly high, 11 1% Most of the offspring of



Fig 1 The 64 chromosomes in spermatogonic metaphase from a male waltzer

these matings was, however firstborns to rather old mothers, therefore 11 1% probably represents a higher than normal perinatal mortality

Waltzers mated to normals gave 236 waltzers and 253 normals this is in excellent agreement with the expected 1 1 ratio There was no significant disproportion between the sexes, showing the gene to be an autosomal one The perinatal mortality in these crosses was moderate, 5.5%. The total frequency of stillborns in the normal-to-normal and in the waltzer-to-normal crosses was 6.8% In literature there are reports with a range from 1 8% to 41.2% perinatal mortality (e.g. Wright, 1922 and 1960 Eaton, 1932 *a, b* Bruce & Parkes, 1948 Rowlands, 1949 and 1955 Goy *et al.*, 1957 Davis & Williamson, 1959 Stuart Paterson, 1962)

There was no difficulty whatsoever in mating waltzers to waltzers. These crosses resulted in 133 dead, 185 waltzers and 106 normal individuals. Amongst the 133 stillborns are the normally perinatal dead, about 7% of the total offspring, that is around 30 animals With correction for this normal mortality the waltzer-to-waltzer crosses yielded about 103 un-accounted-for dead, 185 waltzers and 106 normals, thus well in accordance with the expected 1 2 1 ratio There was no disproportion between the sexes. As described earlier all the waltzers were alike as to behaviour and clinical inner-ear dysfunction, differing only in the age at which the Preyer reflex became unelicitable. The homozygous animals must thus be these 103 (approx.) stillborn individuals.

Accordingly a full description of the heredity in this strain of guinea pigs is a monohybrid dominant Mendelian autosomal gene with a recessive lethal effect and with full expressivity and penetrance.

A study of the litter size in the different groups of crosses showed no significant decrease in the number of offspring per litter in waltzer-to-waltzer crosses. Thus the homozygous fetuses evidently do not to any noticeable extent expire early during the pregnancy Also there was no obvious evidence of an increased number of early abortions among these crosses.

The results of the caesarian sections during the last part of the pregnancy showed that the offspring of waltzer-to-waltzer crosses looked alike and were alive near to birth. The lethal effect on the homozygous animals must accordingly have come into action during a rather narrow perinatal period.

The normal and the waltzing newborns were all able to walk within a few minutes *post partum*. The author has seen some instances where progeny of waltzer-to-waltzer crosses were found very soon after delivery but feebly alive and unable to rise. In those instances the parents actively trampled these to death and devoured them. It seems as if the parents kill all of a litter that do not rise within a short period after delivery

The mechanism of the lethal effect might conceivably be a combination of utter vestibular dysfunction in the homozygous, rendering them unable to rise early and thus initiating their parents instinct to kill abnormal behavioured progeny

In mixed litters there was a tendency for the waltzers to grow slower than the normals obviously the waltzers had greater difficulty in feeding from the mother than normal litter mates. The results in Table 1 show the waltzers tendency to lower birth weight when weighed within 3 days of birth. It is, however not possible to state if this tendency is a consequence of the genotype of the waltzers, i.e. tendency to slightly diminished vitality

ZUSAMMENFASSUNG

Es wurde die genetische Charakteristik eines Stammes waltzender Meerschweinchen mit erblicher progressiver Taubheit studiert. Man fand einen monohybriden dominanten autosomalen mendelnden Erbfaktor mit rezessivem Letaleffekt und mit voller Penetranz und Expressivität

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VESTIBULAR BIDIRECTIONAL SENSITIVITY

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Sixteen spinalized adult cats were stimulated through galvanic polarization monaurally Depolarization produced a horizontal ipsilateral nystagmus, and hyper polarization a horizontal contralateral nystagmus in the intact animals. Thereafter the contralateral labyrinth was destroyed, and the remaining intact laby rinth was stimulated. Depolarization increased the destruction nystagmus, and hyperpolarization decreased the nystagmus to zero, but it was impossible to reverse its direction. It was concluded that it is not possible to cause a nystagmus in both directions from only one semicircular canal.

On the basis of his investigations, Ewald (1892) advanced the theory that the semicircular canals exhibit a bidirectional sensitivity and that an ampullopetal endolymph movement in the horizontal semicircular canal gives a stronger reac tion than an ampullofugal movement. This theory was later confirmed by the experimental work of Löwenstein & Sand (1940) where they were able to show through recording from single fibers, that an isolated semicircular canal at rest has a spontaneous, remarkably constant activity of about 20 impulses/sec. This activity can be caused to increase by ipsilateral rotatory stimulation and to decrease by contralateral rotation. Consequently this type of sensory cell is capable of reacting in different ways on the two sides of a resting position of the cupula. This has been termed bidirectional sensitivity

However it is not only the frequency of discharges in the nerves which is changed during stimulation. By means of microelectrode recording, variations in the D.C. potentials in the endolymph of the semicircular canals were studied during experimental cupula deviation (Trincker 1959) An utriculopetal cupula deviation in the horizontal canal was then invariably found to cause depolarization, and an utriculofugal deviation to result in hyperpolarization.

Löwenstein (1955) has studied the effect of galvanic stimulation on the discharges from the horizontal semicircular canal. He observed that depolarization and hyperpolarization give re spectively increased and decreased discharge frequencies. Thus de and hyper-polarization correspond in their excitatory and inhibitory effects to ipsi- and contra-lateral rotation respectively

Thus the sensory cells in the semicircular canals react in opposite ways on the two sides of the resting position of the cupula, i.e depolarization giving increased and hyperpolarization decreased discharge frequencies. Trincker (1965) supposed that this peripheral bidirectional sensitivity can make motor reflexes run in two opposite directions. According to him the spontaneous activity of the receptors of the semicircular canal should serve as a "zero-point" and increase or decrease of the activity as an indicator of direction of rotation. Since there is no experimental proof substantiating the view that it is possible to run the vestibulo-ocular reflex nystagmus in two opposite direc tions only by stimulation of one semicircular canal we have studied this problem.

MATERIAL AND TECHNIQUE

The experiments were made on 16 adult cats. The animals were anesthetized with ether and

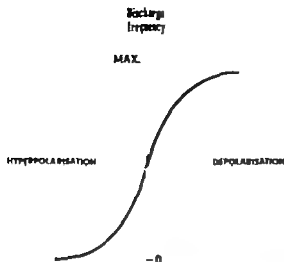


Fig. 1

spinalized between the first and second cervical spinal-cord segments. Before starting the exploration of the middle ear the animals had been treated with local anesthesia (Citanest Exadrin® 0.25%). A vertical incision was made above the ear and the auditory canal was explored and detached from the temporal bone. The auditory ossicles and tympanic muscles were removed. The facial nerve canal was opened and the nerve resected up to the geniculate ganglion. In 8 animals a small fenestration was performed on the ampulla of the horizontal canal and a steel electrode, 0.5 mm diameter, was placed in the openings of the fenestration. In 8 cats no fenestration was performed but the electrode was only placed on the small bluish spot on the medial wall of the facial canal, which is just outside the horizontal ampulla. The indifferent electrode was placed subcutaneously on the head of the cat.

The stimulation was applied through galvanic polarization monaurally with a Grass-stimulator and a Grass Constant Current Unit with a resistance coupled parallel with the cat on the output of the latter in order to decrease the current through the animal down to minimum 1 μA. The horizontal semicircular canal was exposed to either depolarization or hyperpolarization, and the resulting eye movements were studied. Thereafter the contralateral labyrinth was destroyed either surgically or by cauterization and then the same experiment was made on the remaining intact labyrinth.

RESULTS

Depolarization in intact animals invariably caused horizontal ipsilateral nystagmus. The threshold for this reaction was about 0.2–0.8 mA and on the whole irrespective of whether fenestration was performed or the ampulla indirectly stimulated through the bone. Hyperpolarization, on the other hand, gave a horizontal contralateral nystagmus. Increase in the strength of stimulation always resulted in increased nystagmus intensity.

After surgical destruction of the contralateral labyrinth, a lively destruction nystagmus, directed towards the normal ear was observed in the animals. During electrical destruction by cauterization the cats first exhibited a very lively nystagmus towards the cauterized ear but after a few seconds its direction was reversed which was a proof of the destruction. Depolarization of the remaining intact labyrinth gave rise to an increase in the intensity of the established destruction nystagmus. If on the other hand, hyperpolarization was induced the destruction nystagmus was totally inhibited at about 0.8 mA. If the strength of the current was then slowly increased, even up to 20 mA, nystagmus in a reverse direction was never obtained. Instead, the strength of the current resulted in destroying also this labyrinth which was shown by the disappearance of the destruction nystagmus and by the impossibility of further stimulating the labyrinth by d-polarization.

DISCUSSION

The investigation has shown that in the intact animal it is possible by galvanic polarization to cause a nystagmus whose direction and intensity are dependent on both the polarity and the strength of the polarization. Löwenstein (1956) has shown that the bidirectional sensitivity of the semicircular canal has a charac

teristic S-formed curve of action-potential frequency which is very similar to that of an electronic triode valve with the "zero-point" in the middle of the curve Fig. 1 shows the characteristic of such a curve From this it is evident that depolarization increases activity and hyperpolarization diminishes it towards zero.

The peripheral bidirectional sensitivity implies that the sense organ, through its discharge frequency provides information on which side of its resting position the cupula is actually deviating. If this bidirectional sensitivity can also give rise to motor reflexes of the type of nystagmus in opposite directions, then stimulation of the semicircular canal with opposite polarities would be expected to give nystagmus of opposite directions. This also proves to be the case if the semicircular canal is polarized on one side but only provided that the contralateral labyrinth is intact. If the latter is destroyed, a destruction nystagmus towards the intact labyrinth is obtained but neither depolarization nor hyperpolarization of the intact labyrinth is able to reverse this nystagmus. It is only possible to make the nystagmus reaction run in one direction along the characteristic of the above-mentioned curve from maximum during depolarization towards zero during hyperpolarization. Consequently it must be concluded that it is not possible to cause a nystagmus in both directions from only one semicircular canal. That after a certain time it is again possible to cause nystagmus in both directions must be due to other compensatory factors which are centrally released. These factors will be dealt with in a later paper

ZUSAMMENFASSUNG

Sechzehn spinalisierte erwachsene Katzen wurden durch monaurale galvanische Polarization stimuliert. Die Depolarization gab einen horizontalen ipsilateralen Nystagmus und die Hyperpolarization einen horizontalen kontralateralen Nystagmus in normalen Tieren. Danach wurde der kontralaterale Labyrinth destruiert und der noch vorhandene Labyrinth wurde stimuliert. Die Depolarization vergrösserte den Destruktionsnystagmus und der Hyperpolarization verkleinerte den Nystagmus bis Null, aber es war unmöglich seine Richtung zu ändern. Die Folgerung ist, dass es nicht möglich ist einen Nystagmus in beiden Richtungen nur von einen Bogengang herbeizuführen.

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PSYCHOPHYSICAL SCALING OF ELECTRIC TASTE

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By means of the method of magnitude estimation the psychophysical function of electric taste was de termined in 6 normal subjects. With purely anodal tongue stimulation the subjective intensity of the electric stimulus grows as a power function of the physical stimulus intensity up at least 300 μA. The value of the exponent n varied individually from 0.7 1.8 with an average of 1 1

The relationship between physical and subjec tive stimulus intensity has—for more than a century—been one of the most intriguing problems in sensory physiology The range of intensities to which a sense organ reacts is limited by the absolute threshold and a somewhat diffuse maximum level such as the pain threshold It is usually agreed that within this range there is a non-linear relation between physical and subjective stimulus intensity The exact form of the function, however has been subject to much disagreement. Where discrimination is concerned, it is well-known that Weber (1834) found the just noticeable difference" (jnd) roughly proportional to the stimulus level ($\Delta I/I = k$) On a purely theoretical basis, Fechner (1860) applied Weber's law to the psychophysical function, postulating that equally often noticed differences (jnd) are of equal subjective intensity or in other words, that a scale of subjective intensity can be con structed by adding jnd's. In the nineteen-thirties, Stevens (1936) initiated a more ex perimental approach to psychophysics and it was soon demonstrated that the subjective intensity of a jnd is not constant but grows with the level of intensity As the true form of the psychophysical function Stevens (1957) suggests a power function ("power law"), $S = k \times R$ in which S is the stimulus intensity R the subjective intensity and n an exponent, which usually varies from 0.5–2.0 Plotted in a log-log-diagram this function follows a straight line, the slope of which indicates the exponent n During the last three decades, an extensive literature including almost all sensory modalities has provided ample evidence in favour of Stevens theory

The psychophysical function of chemical taste has been investigated by several authors. Lewis (1948) used the so-called "method of ratio production" and found that the intensity functions for the four traditional taste qualities obey the power law Based on cross-qualitative matching experiments Beebe-Center & Waddell (1948) constructed a general psychological *scale of chemical taste. In further consequence* of this, Hinchcliffe (1958) recommended the use of a new method of clinical taste examination. For each of the four taste qualities he selected ten concentrations distributed according to the intensity function found by Beebe-Center & Waddell. The size of the exponent n was evaluated for salt by Ekman (1961) and found to be 1.59 Stevens (1961) gives the value 1 3 for sucrose and salt and 0 8 for saccharine but no details of the experiments are reported

The intensity function of electric taste was investigated by Heimbrecht (1968) and Jauhiainen *et al* (1967) As neither of these au-

Table 1 *The stimulus intensities used in the present study and by Jauhiainen et al*

Føns	30–54–96–170–300 μA
Jauhiainen et al.	30–50–90–120–200 μA

thors, however used purely anodal, constant current stimulation, their results cannot be applied to the method of stimulation employed by Krarup (1958) Thus, Helmbrecht stimulated with series of rectangular anodal impulses, the duration of the impulses varying from 0 1–3 msec and the frequency from 100–400/sec With the resulting stimulus patterns he was able to produce taste sensations comparable to the four basic taste qualities. The intensity function, registered by means of a "ratio production method" was found to obey Stevens power law the average value of the exponent *n* being equal to 1 075 Jauhiainen *et al.* preferred the "method of magnitude estimation (Stevens, 1961) and registered the numerical estimates of five different stimulus intensities (Table 1) Sixty estimates of each intensity were obtained in each of six subjects. The results are given in Fig. 1 B Up to 120μA the function is a straight line with a slope corresponding to $n = 1.2$. Above 120 μA the slope decreases to $n = 0.5$ As an explanation of this change of the slope, Jauhiainen *et al* suggest that the maximum of subjective taste sensation is reached or that two receptor systems,

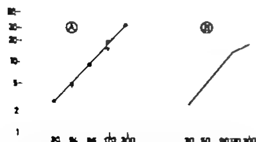


Fig. 1 (A) The psychophysical function determined in the present study A best fitting straight line was drawn through the means (circles). The medians are indicated by crosses. (B). The results obtained by Jauhiainen *et al.* The slope of the function decreases at 120 μA.

taste as well as tactile receptors, are stimulated.

This alternative can be further evaluated if the electrode used by Jauhiainen *et al* is taken into consideration. They used a double electrode, the anode and the cathode both being placed on the tongue surface The two electrodes were made of stainless steel with flat, circular contact areas, 5 mm in diameter and they were separated 1 cm from each other The direct contact, however between a bipolar electrode and two different receptor systems makes the stimulatory situation rather complicated. The available facts, relevant to this problem shall therefore be briefly summarized.

1 Anodal stimulation of taste receptors gives the lowest threshold, normally of the order of 10μA. Discrimination experiments (Føns & Osterhammel, 1968) seem to indicate that the subjective intensity of the anodal taste sensation grows with the stimulus intensity up to at least 300 μA, which is the maximum intensity of Krarup's electrogustometer (Krarup, 1958)

2 Anodal stimulation of tactile receptors innervated by the trigeminal nerve branches to the anterior two-thirds of the tongue does not seem to occur This can easily be demonstrated in patients with hemiageusia due to middle ear surgery who exhibit a convenient combination of preserved tactile sensation and abolished taste sensation. These patients do not respond to an anodal current of 300 μA.

3 Cathodal stimulation of taste receptors shows a threshold which is usually 3–10 times higher than that of anodal stimulation, but often less than 100 μA. It is therefore quite possible that Jauhiainen's double, bipolar electrode causes a pure, anodal taste stimulation at low intensities and a mixed, anodal/cathodal taste stimulation at higher intensities.

4 Cathodal stimulation of tactile receptors can sometimes be seen at intensities down to 100 μA. It can be demonstrated in patients with hemiageusia, but in normal subjects tactile stimulation at moderate intensities can also be demonstrated by applying the cur

the lip, where taste receptors are absent. As the tactile sensation is almost impossible to distinguish from the pure taste sensation, the presence of the cathode on the tongue involves a risk of misinterpreting the result of the taste examination.

The described polarity relationships strongly suggest that the findings of Jauhiainen *et al* —particularly the change of the slope at 120 μA—may be due to the use of a bipolar tongue electrode. It has therefore been the purpose of the present study to investigate the psychophysical function of electric taste using purely anodal constant current stimulation.

METHOD

Stimulator

Two constant current generators were used, one set at the constant standard stimulus intensity the other at the variable test stimulus intensities. The stimulus- and interval duration as well as the sequence of standard and test stimuli were automatically controlled by a unit interposed between the two stimulators and the tongue electrode

lectrodes

e anode was a flat, circular steel electrode, mm in diameter (Krarup, 1958) which the subject held in position on the tip of the tongue As cathode was used a 4 × 4 cm chromium plated brass electrode placed on the wrist with a piece of gauze underneath soaked in physiological saline solution

Stimulus intensities

Five stimulus intensities (Table 1) were distributed logarithmically over the range from 30–300 μA The medium intensity 96 μA, was also used as standard intensity its subjective intensity being defined as 10" The stimulus intensities used by Jauhiainen *et al* (1967) are included for comparison in Table 1

Presentation

Each estimation of a certain test stimulus was given after a series of standard and test stimuli presented alternately in the order S P S P P S P S The impulses were rectangular and 500 msec in duration (Føns & Osterhammel 1968) The first three and the last three intervals were of 6 sec duration, the middle one, during which the sequence was reversed, approximately twice as long. Sixty estimates of each of the five intensities were obtained in each subject the order of presentation being randomized.

Table 2. *Average results obtained at the five different stimulus intensities calculated as arithmetic means as well as medians*

The standard deviations corresponding to the means are given in per cent

Stimulus intensity (μA)	30	54	96	170	300
Mean	2.9	5.3	10.2	20..	35.4
Standard deviation (%)	85	48	76	51	59
Median	1.7	4.9	9.5	16.5	28.0

Test subjects

Six subjects, all young students, were tested in 6–8 sessions of approximately one hour

Instructions

The subjects were instructed to estimate the magnitude of the test stimulus in relation to the standard stimulus which was called 10" (Stevens, 1961) Any numerical value, fractions included could be used The subjects were not informed about the upper and lower limits of the intensity range, nor of the number of different test stimuli. They were told that standard- and test stimulus might sometimes be identical.

RESULTS

For each of the five test stimulus intensities the arithmetic mean of the 360 registrations was calculated. The results are given numerically in Table 2 and graphically in Fig. 1 A. In the log-log-diagram the psychophysical

Table 3 *The slope of the individual and average curves represented by the numerical value of the exponent n*

Subject	1	2	3	4	5	6	Average
n-value	1.8	0.8	1.1	0.7	0.7	1.8	1.1

function follows a practically straight line with a slope corresponding to $n = 1.1$ The slope of the individual curves was also calculated and the values of the exponent n are given in Table 3

In Fig. 1 A the straight line was drawn along points corresponding to the arithmetic means. This was done in order to compare the results with those of Jauhiainen *et al* shown in Fig. 1 B Now the arithmetic mean is only representative if the distribution is normal This condition was not quite fulfilled as the distributions showed a slight but constant, unsymmetrical extension toward the higher values. In such cases, Stevens (1957) suggests the calculation of medians instead of means. As expected the medians were all slightly lower than the means, the difference being roughly proportional to the distance from the standard stimulus intensity (Fig. 1 A) This downward concavity of a curve through the medians was due to the combination of a slightly skewed distribution and a standard deviation which is smallest around the standard stimulus intensity (Table 2) A best fitting, straight line through the medians, however would give the same slope as the curve corresponding to the means.

DISCUSSION

As the most important conclusion of the present study it can be stated that the psychophysical function of electric taste as plotted in a log-log-diagram follows a straight line up to at least 300 μA This is in accordance with Stevens power law but counts against Jauhiainen s theory that the maximum of the subjective intensity of electric taste is reached around 120 μA.

The individual differences were rather pronounced. As shown in Table 2, the slope varied from $n = 1.8$ to $n = 0.7$ This means that the average slope, $n = 1.1$ cannot be considered a general psychological scale of electric taste The problem of choosing a scale unit for the electrogustometer has therefore not found an unquestionable solution in the present results. Clinical threshold determinations for which the electrogustometer is primarily used, are probably more closely related to the discrimination ability than to the psychophysical scaling. The logarithmic scale recommended in a previous publication represents a reasonably good approximation to the discrimination ability in electric taste and has therefore been chosen for a new electrogustometer which is being developed in our clinic.

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ZUSAMMENFASSUNG

Die psychophysische Funktion des elektrischen Geschmacks wurde mittels einer magnitude estimation Methode untersucht. Wenn nur die Anode auf der Zunge angelegt wird, steigt die subjektive Empfindungsgrosse als eine Potenzfunktion der Reizstarke bis mindestens 300 μA. Der Exponent dieser Potenzfunktion liegt zwischen 0,7 und 1,8 im Durchschnitt auf 1 1

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RESEKTION DER NASENSCHEIDEWAND IM KINDESALTER

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Auf Grund der Letzten Forschungen über die Nasefunktion schlagt der Verfasser rationelle operative Eingriffe in der Kindernase vor wo die Obstruktion durch Fraktur oder Septumdeviation verursacht wurde. Durch die spirometrische Analyse fand man dass sich die respiratorische Lungenfunktion in beträchtlicher Weise nach Obstruktionsentfernung verbessert hat.

Indikationen und Methoden der chirurgischen Eingriffe die zur Zeit Killians und Denkers in der Behandlung von Erkrankungen der Nase und Nasennebenhöhlen souverän geherrscht haben, sind heute — dank der neueren Erkenntnisse der Physiologie der Nase und Respirationsschleimhaut — nicht nur bei Erwachsenen, sondern auch bei Kindern verändert.

Wir müssen die bestehende Auffassung ändern, dass operative Eingriffe in der Nase schädlich seien, solange sie wächst und sich entwickelt. Wir sind es gewohnt, die Septumresektion erst nach der Pubertät auszuführen das ist frühestens zwischen 15 und 16 Jahren.

Wenn man ausserdem bedenkt, dass das Atmen durch die Nase die erste Bedingung für eine normale Entwicklung der Lunge und ihrer Elastizität ist, wird man leicht die Folgen begreifen, welche eine Nasenobstruktion durch einenhalb Dezennien auf die physische und psychische Entwicklung des Kindes hinterlassen würde.

Zu den wichtigsten Aufgaben der Nase gehört, dass sie wärmt, filtriert und die Luft befeuchtet, sowie die Atmung vertieft und auf diese Weise optimale Bedingungen für den Gasaustausch auf Alveolenniveau schafft. Bei der Obstruktion hervorgerufen durch Deviation oder Septumfraktur Verdickung der Schleimhaut, entzündlicher oder allergischer Art, Polypenwachstum oder bei reflektorischer Vasodilatation erhöht sich die Resistenz der Luftströmung. Die Folge davon ist eine verstärkte Arbeit der Atmungsmuskulatur oder der Mundatmung, was die Lungenventilation verschlechtert, die Alkalireserve im Blut verringert und negativ auf den allgemeinen Stoffwechsel einwirkt.

Viele Forscher haben die Nasenobstruktion als Faktor des gestörten Atmungsmechanismus studiert, aber bis heute is diese noch nicht voll aufgeklärt. Die Mittel, die uns zur Funktionsprüfung der Nase dienen, haben sich parallel mit der besseren Erkenntnis und Erfahrung ihrer Physiologie geändert. Es besteht eine enge Verbundenheit nicht nur mit den anatomisch nahen Organen, Ohr Nasennebenhöhlen und Hals, sondern auch mit den entfernteren wie den Lungen, den Drüsen mit innerer Sekretion, den parenchymatosen Organen sowie dem vegetativen Nervensystem. So wurde Ende des vergangenen Jahrhunderts die Durchgängigkeit der Nase mit einem Quecksilbermanometer geprüft. In unserem Lande haben Sercer (1962 und 1965) und Čurković (1937) in der Zeit zwischen den beiden Weltkriegen Wassermanometer konstruiert, bei welchen die Oszillationen des positiven und negativen Druckes beim Atmen besser ersichtlich er

neuesten Zeit ist die Rhinomanometrie nach Cottle mit elektronischer Registration dazu geeignet, alle Unregelmässigkeiten bei der Atmung anzuzeigen. Sie hat sich durch ihre Genauigkeit der Elektrokardiographie oder Audiometrie genähert. Für den täglichen Gebrauch ist sie heute jedoch noch nicht verwendbar

Es sind auch viele Versuche unternommen worden, das Verhältnis zwischen Luftströmung und Druck in den Nasenwegen zu bewerten, d. h. den Widerstand zu messen, welchen die Nase der Luft bei Ein- und Ausatmung leistet. Während der Atmung durch die Nase ist der Gesamtdruck in den Lungenalveolen gleich der Gesamtsumme des Druckes in der Nase plus des Druckes in den unteren Atmungswegen. So entfällt bei der Geschwindigkeit der Luft von 10 l/min auf den Widerstand in der Nase 50% des Gesamtwiderstandes während er bei 40 l/min 60% beträgt. Je grösser die Luftzufuhr ist, desto mehr beteiligt sich die Nase an den Gesamtwiderstand im Vergleich mit den unteren Atmungswegen.

In neuerer Zeit hat Ogura mit seinen Mitarbeitern (1966) das Verhältnis der oberen Atmungswege zu den Lungen untersucht. Er hat gesunde Personen und Personen mit Nasenobstruktion verschiedener Grade und Ursachen geprüft. Den Lungenwiderstand hat er mit Hilfe eines Ballons gemessen, den er in die Speiseröhre einführt. Im Vergleich mit dem Widerstand gemessen durch den Mund, fand er dass sich dieser in der Nase durch Erhöhung der Obstruktion bis zu 500% vergrössern kann. Wurde die Atmungsstörung durch Operation oder durch Vasokonstriktoren entfernt so verringerte sich der Lungenwiderstand. Ogura ist daher mit seinen Mitarbeitern zu dem Ergebnis gekommen, dass die Messung der Ventilationsmöglichkeiten der Lunge durch den Mund mit dem Grad der Nasenobstruktion steigen oder fallen kann. Es ist schwer festzustellen, ob solche Befunde durch Veränderungen, die durch Kongestion oder Fibrose am Lungenparenchym hervorgerufen werden, bedingt sind, weil die geprüften Personen gesunde Lungen hatten. Es besteht die Möglichkeit, dass das Nasen-Lungen-Nerven-System einen Reflexbogen hat, und dass eine ständig verstopfte Nase auf irgendeine Reflexart die Lungenfunktion stört. Auf die verringerte Funktionsmöglichkeit der Lungen könnte auch die veränderte chemische Zusammensetzung der Gase auf Alveolenniveau einwirken. Oguras Annahme dass die Ergebnisse der Untersuchungen der Lungenfunktion gemessen durch den Mund von der Durchgängigkeit der Nase abhängig sind, haben wir benützt, um einen eventuellen Vorteil der Septumkorrektion im Kindesalter an der Lungenfunktion zu beweisen

Das Grundprinzip bei der Behandlung aller Nasenkrankheiten müsste darin bestehen den physiologischen und funktionellen Mechanismus dieses Organes wieder herzustellen. Hingegen ist die Beurteilung der chirurgischen Eingriffe an der Nasenpyramide und Nasenscheidewand nicht so leicht, weil auf diesem Gebiete neben anatomischen und physiologischen Erfolgen auch kosmetische ästhetische und psychische Momente eine Rolle spielen. Eine subjektive Bewertung des Arztes und besonders des Kranken, kann, was die Funktion betrifft, für die Statistik nicht zuverlässig sein. Von der Testierung vielfacher Funktionen haben wir uns nur auf die Suche einer eventuellen Gesetzmässigkeit des Verhaltens zwischen der Durchgängigkeit der Nase und dem funktionellen Zustand der Lunge den wir durch Spirometrie geprüft haben begrenzt. Wenn man bedenkt, was sowohl durch Versuche als auch klinisch bestätigt ist, dass die erschwerte Atmung durch die Nase schädlich auf die Atmungsfunktion der Lunge wirkt, dann würde durch spirometrische Untersuchungen vor und nach der Obstruktionsentfernung eine objektive Bewertung sowie der Vorteil eines chirurgischen Eingriffes gerechtfertigt sein.

Nasenoperationen im Kindesalter gehören sicher zu den delikatesten rhinologischen Eingriffen. Schon aus oben angeführten Gründen müsste die konservative Heilung der Nasenob-

struktion unterlassen werden. Besonders Verletzungen einer Kindernase führen, wenn sie nicht radikaler behandelt wird, nicht nur zu ihrer Deformation, sondern es wird auch mehr oder weniger ihre Funktion geschädigt. Bei der Erkenntnis dass möglicherweise eine gestörte Funktion vorliege, ermutigt uns für eine aktive Behandlung auch die Tatsache, dass sich die Nasenstrukturen nicht gleichmässig intensiv im ganzen Kindesalter entwickelt. Es ist bekannt, dass die Nase am schnellsten in den Jahren von 1–7 und 11–16 wächst. Zwischen dem 7 und 11 Jahr herrscht in der Entwicklung ein relativer Stillstand. Wenn wir uns bei unseren Eingriffen an folgendes Prinzip halten, aus der Nase nur das zu entfernen, was der Atmung im Wege steht, oder die Teile, die entweder durch Trauma oder angeboren disloциert sind, auf ihren ursprünglichen Platz zu stellen — also im strengsten Sinne des Wortes eine physiologische Behandlung anzuwenden — könnten wir im voraus alle Einwände verwerfen, die sich auf eventuelle später entstandene Mutilationen in der Entwicklung der Nase und des Gesichtes zeigen würden. In Jugoslawien haben Gošić (1951) und Krajina (1965), die diese Ideen publiziert haben, besondere Verdienste erworben.

Nach Fischer (1957) unterscheiden sich die Indikationen zur Nasenscheideresektion im Kindesalter nicht sehr von jenen bei Erwachsenen, es sind dies: Nasenobstruktion, Ohrenkrankungen, Nasenblutung, Kopfschmerzen, Bronchialasthma und Verletzungen. Seine Statistiken für ein Dreijahresintervall zeigen, dass er 242 Nasenseptumresektionen davon 55 bei Kindern ausgeführt hat. Dieses stellt nach unserer Meinung für eine Stadt von 35 000 Einwohnern eine zu breite Indikation dar

In unserer Kasuistik haben wir uns streng nur an jene Fälle gehalten, bei denen die Atmung durch die Nase derart erschwert war dass sich die Störungen auch nach Entfernung der Rachentonsille nicht gebessert haben. Aus Tabelle 1 ist ersichtlich, dass vir in den letzten fünf Jahren 1 8–3 8% Operationen an der Nasenscheidewand von den Gesamteingriffen hatten, was jährlich im Durchschnitt ca 44 ausmacht. Im gleichen Zeitraum haben wir nur 17 Operationen an der Nasenscheidewand bei Kindern bis zu 15 Jahren ausgeführt. Auf Tabelle 2 sehen wir Angaben aus Krankheitsgeschichten. In der Mehrzahl der Fälle haben die Eltern nicht gewusst, seit wann das Kind schwer durch die Nase atmet, geben einen ungefähren Zeitpunkt an, an den sie sich erinnern können. Bei 7 Fällen waren Verletzungen der Grund der Obstruktion. Interessant ist die Tatsache, dass wir 13 Knaben und 4 Mädchen operiert haben. Kann man die Frakturen der Nasenscheidewand vielleicht der grösseren Lebhaftigkeit der Knaben und dadurch der Möglichkeit häufigerer Verletzungen zuschreiben?

Alle Eingriffe haben wir unter endotrachealer Anesthäsie mit Äther und lokaler Infiltration von Xylocain und Suprarenin ausgeführt.

Tabelle 1

Septumresektionen i den letzten 5 Jahren

Jahr	1963	1964	1965	1966	1967
Gesamtzahl der op. Eingriffe	1762	1986	1721	1431	1648
Septumresektionen	32	59	44	47	36
Resektion unter 15 Jahren	0	2	4	7	4
Prozent	1,8	3 1	2	3 8	2,4

Tabelle 2.

Anamnese	
1 Zeitpunkt, von dem an nach Erinnerung der Eltern, das Kind schwer durch die Nase atmete	10 Fälle
	7 Fälle
2. Nasenverletzung	
Knaben	13 Fälle
Mädchen	4 Fälle
Lokalbefund	
Septumfraktur	7 Fälle
Subluxation	6 Fälle
Verdicktes Septum	2 Fälle
Crista oder Spina	2 Fälle

Spirometrischer Befund Nr 1

Vor und Zuname: Alma Rusnjak. Datum 19 VIII 1967
Geburtsjahr: 1957 Grösse 152 cm. Lauf Nr 154/67
Diagnose: Fractura septi nasi. Körpergewicht. 42 kg.
Luftdruck 763 mm Hg. Zimmertemperatur: 26°C.

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	22	
Minutenvolumen		6600	
Sauerstoffverbrauch		120	
Vitalkapazität	2400	2688	112
Ventilations-equivalent	2,4 ± 0,6	5,5	
Maximalminuten-volumen in Lt.	60	59	98
Tiffeneautest in ccm		2100	
Tiffeneautest in VK	83 %	78 %	
Luftgeschwindigkeits-index	1,0 ± 0,2	1,87	

Meinung und Beschluss: Spyrometrischer Befund in Normalgrenzen. (Dr P Cukon.)

Spirometrischer Befund Nr 2

Vor und Zuname: Vladimir Sinožić. Datum 23.II.1968
Geburtsjahr: 1956. Grösse: 150 cm. Lauf Nr 32/68.
Diagnose: Status post resectionem septi nasi.
Körpergewicht 59 kg. Luftdruck 762 mm Hg. Zimmertemperatur: 22°C.

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	20	
Minutenvolumen		7800	
Sauerstoffverbrauch		220	
Vitalkapazität	2550	2320	92
Ventilations-äquivalent	2,4 ± 0,6	3,5	
Maximalminuten-volumen in Lt.	64	59	92
Tiffeneautest in ccm		1930	
Tiffeneautest in % VK	83 %	83 %	
Luftgeschwindigkeits-index	1,0 ± 0,2	1,0 %	

Meinung und Beschluss. Spyrometrischer Befund in Normalgrenzen. (Dr P C kon.)

Wie wir schon erwähnten haben wir uns an das Prinzip einer möglichst konservativen chirurgischen Behandlung gehalten und haben nur in 2 Fällen die klassische Resektion nach Killian angewendet. Es gelang uns bei 8 Kindern die spirometrische Analyse vor und sechs Monate nach der Operation durchzuführen.

Wir müssen betonen, dass während der Spirometrie viel Geduld und enge Zusammenarbeit zwischen dem Kind und der Schwester notwendig sind, um zu glaubwürdige Ergebnisse zu kommen. Ein Intervall on 6 Monaten halten wir für eine eventuelle Besserung der Lungenfunktion für ausreichend.

Spirometrischer Befund Nr 1A

Vor und Zuname Alma Rusnjak. Datum 18.III 1968.
Geburtsjahr: 1957 Grösse: 157 cm. Lauf N 48/68.
Diagnose: St post resectionem septi nasi.
Körpergewicht 4 kg.
Luftdruck 763 mm Hg. Zimmertemperatur 26°C

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	18	
Minutenvolumen		4320	
Sauerstoffverbrauch		170	
Vitalkapazität	2650	2840	107
Ventilations-equivalent	2,4 0,6	2,5	
Maximalminuten-volumen in Lt.	67	60	89
Tiffeneautest in ccm		1930	
Tiffeneautest in VK		89	
Luftgeschwindigkeits-index	1,0 ± 0..	0,83	

Meinung und Beschluss Spyrometrischer Befund in Normalgrenzen. (Dr P Cukon.)

Spirometrischer Befund Nr 2A

Vor und Zuname: Vladimir Sinožić. Datum. 21 VII 1967.
Geburtsjahr: 1956. Grösse 147 cm. Lauf Nr 135/67
Diagnose: Deviatio septi nasi. Körpergewicht: 40 kg
Luftdruck 763 mm Hg Zimmertemperatur: 28°C

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	22	
Minutenvolumen		8600	
Sauerstoffverbrauch		256	
Vitalkapazität	2450	2140	88
Ventilations-äquivalent	2,4 ± 0,6	3,4	
Maximalminuten-volumen in Lt	62	55	89
Tiffeneautest in ccm		1830	
Tiffeneautest in % VK	83	86	
Luftgeschwindigkeits-index	1 0 ± 0,2	1,01	

Meinung und Beschluss. Es bestehen geringere Ventilationsstörungen restriktiver Art. (Dr P Cukon.)

Spirometrischer Befund Nr 3

Vor und Zuname: Nadija Arman. Datum. 15.IX.1967
Geburtsjahr: 1959 Grosse 144 cm. Lauf Nr 172/67
Diagnose: Deviatio septi nasi. Körpergewicht. 32 kg.
Luftdruck 760 mm Hg. Zimmertemperatur: 24°C.

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	38	
Minutenvolumen		6840	
Sauerstoffverbrauch		140	
Vitalkapazität	2100	1780	85
Ventilations-äquivalent	2,4±0,6	4,8	
Maximalminuten-volumen in Lt.	52	38	73
Tiffeneautest in ccm		1290	
Tiffeneautest in VK	83 %	72 %	
Luftgeschwindigkeits-index	1,0±0,2	0,86 %	

Meinung und Beschluss Nach gefundenen Werten handelt es sich um Ventilationsinsuffizienz restriktiver Art. (Dr P Cukon.)

Spirometrischer Befund Nr 4

Vor und Zuname: Marino C kerl. Datum. 20.IV 1967
Geburtsjahr: 1957 Grösse: 145 cm. Lauf Nr.. 59 67
Diagnose: Fractura septi nasi. Korpergewicht. 32 kg.
Luftdruck. 763 mm Hg. Zimmertemperatur: 15°C

Benennung	Normal-befund	Gefundener Wert	Prozent d Normale
Atmungsfrequenz	16	31	
Minutenvolumen		14000	
Sauerstoffverbrauch		240	
Vitalkapazität	2350	2460	105
Ventilations-äquivalent	2,4±0,6	5,8	
Maximalminuten-volumen in Lt.	59	53	90
Tiffeneautest in ccm		1850	
Tiffeneautest in ~ VK	83	75	
Luftgeschwindigkeits-index	1,0±0,2	0,86	

Meinung und Beschluss. Spyrometrischer Befund deutet uf Beginn einer Ventilationsinsuffizienz obstruktiver Art leichteren Grades. (Dr P Cukon.)

Ergebnise der Spirometrie. In 3 Fällen war der Befund vor und nach dem Eingriff normal (Befund 1 und 1 A) In 4 Fällen fand man vor der Resektion verminderte spirometrische Werte, die für die Ventilationsinsuffiziens restriktiver Art sprachen, und 6 Monate nach dem Eingriff war der spirometrische Befund in Normalgrenzen (Befund 2, 3 und 2 A, 3 A) Bei einem Knaben, bei dem neben einer ausgesprochenen Fraktur der Nasenscheidewand

Spirometrischer Befund Nr 3 A

Vor und Zuname: Nadija Arman. Datum 18.III.1968
Geburtsjahr: 1959 Grosse: 147 cm. Lauf. N 47/68.
Diagnose: Status post resectionem septi nasi.
Korpergewicht. 37 kg. Luftdruck. 762 mm Hg. Zimmer temperatur: 21°C.

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	30	
Minutenvolumen		7200	
Sauerstoffverbrauch		170	
Vitalkapazität	2150	2000	93
Ventilations-äquivalent	2,4±0,6	4,2	
Maximalminuten-volumen in Lt.	54	50	93
Tiffeneautest in ccm		1860	
Tiffeneautest in % VK		90 %	
Luftgeschwindigkeits-index	1,0±0,2	1,0	

Meinung und Beschluss: Spyrometrischer Befund in Normalgrenzen. (Dr P Cukon.)

Spirometrischer Befund Nr 4 A

Vor und Zuname: Marino Cukeri. Datum. 25.XII.1967
Geburtsjahr: 1957 Grösse: 148 cm. Lauf. N 255/67
Diagnose: Status post resectionem septi nasi.
Körpergewicht: 36 kg. Luftdruck: 751 mm Hg. Zimmertemperatur: 23°C.

Benennung	Normal-befund	Gefundener Wert	Prozent d. Normale
Atmungsfrequenz	16	34	
Minutenvolumen		7560	
Sauerstoffverbrauch		530	
Vitalkapazität	2450	3140	128
Ventilations-äquivalent	2,4±0,6	1 4	
Maximalminuten-volumen in Lt.	62	40	64
Tiffeneautest in ccm		1330	
Tiffeneautest in % VK	83 %	43 %	
Luftgeschwindigkeits-index	1,0±0,2	0,50	

Meinung und Beschluss: Nach erhaltenen Worten handelt es sich um Ventilationsinsuffizienz obstruktiver Art schweren Grades. Es wäre notwendig eine Spyrometrie mit Aleudrintest zu wiederholen. (Dr P Cukon.)

auch Zeichen einer vasomotorischen Rhinitis bestanden, war der spirometrische Befund vor der Operation ausgesprochen pathologisch Nach der Operation entwickelte sich Bronchialasthma und der spirometrische Befund bewegte sich noch mehr in pathologischer Richtung (Befund 4 und 4 A) Dieses Beispiel ist uns ein Beweis, dass wir bei einer Auswahl der Fälle und Aufstellung der Indikationen sehr vorsichtig sein müssen. In der Nase als reflektorisches Organ und besonders auf ihrer Respirationsschleimhaut können und dürfen wir nicht immer die Obstruktion auf chirurgischem Wege erledigen, wobei wir die Grundursache, die zur erschwerten Atmung geführt hat, vergessen und umgehen.

KONKLUSION

Der Nasenuntersuchung bei Atmungsbeschwerden im Kindesalter müssen wir mehr Aufmerksamkeit widmen. Wenn wir überzeugt sind, dass der Grund in einer Fraktur oder Deviation der Nasenscheidewand liegt, sollten wir nicht von einer Operation absehen, die in erster Linie funktional sein muss, d. h. nur jene Teile des Septums zu entfernen, die das Hindernis er Luftströmung durch die Nase darstellen. Der Eingriff ist heikel und dürfte nur von em erfahrenen Chirurg ausgeführt werden. Es ist sicher dass in streng indizierten Fällen auch die funktionalen und kosmetischen Ergebnisse gut sein werden.

RÉSUMÉ

Sur la base des recherches récentes de la fonction nasale, l'auteur propose l'intervention chirurgicale dans le nez, chez les enfants ou l'obstruction a été provoquée par fracture ou déviation de la cloison. Les analyses spirométriques ont montré que la fonction respiratoire du poumon après l'élimination de l'obstruction nasale a été notamment améliorée.

SUMMARY

According to the recent research about the function of nose, the authors suggest rational surgical operations in the nose of children, where the obstruction is caused by septal deviation or fracture. Spirometric analysis found out that respiratory function of lungs after removing of obstruction was considerably improved.

Für die erteilte Hilfe in Spirometrieanalyse bin ich Herrn Chefstellvertreter der Lungenabteilung Dr Petar Cukon zu tiefstem Dank verpflichtet.

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DIE MIKROLARYNGOSKOPIE UNTER NEUROLEPTANALGESIE MIT EINEM SELBSTKONSTRUIERTEN ENDOSKOP NACH HASLINGER

H Eickhoff

Aus der Abteilung für Hals-Nasen-Ohren-Krankheiten der Medizinischen Fakultät an der RWTH Aachen DBR

Eingegangen am 2. Oktober 1969

Die Neuroleptanalgesie ohne zusätzliche Intubationsnarkose bietet bei der Mikrolaryngoskopie und endolaryngealen Mikrochirurgie gegenüber anderen Narkoseverfahren für Patienten und Operateur deutliche Vorteile. Insbesondere durch Fortfall des Intubationsschlauches sind umfassender Einblick in den Kehlkopf und unbehindertes mikrochirurgisches Arbeiten möglich. In unserer Klinik wird der Kehlkopf mit einem auf die speziellen Bedürfnisse abgewandelten Haslinger-Endoskop eingestellt. Die Vorzüge dieses Endoskops finden ihre Erklärung in der idealen Ausnutzung der Mechanik von Arm und Hand.

Durch die Mikrolaryngoskopie erfahren die diagnostischen und therapeutischen Möglichkeiten unseres Fachgebietes eine wesentliche Bereicherung.

Insbesondere von Kleinsasser (1968) wurde die Methode der endolaryngealen Mikrodiagnose und -chirurgie zu großer Perfektion entwickelt. Nach eigenen Erfahrungen mit über 300 Mikrolaryngoskopien möchten wir hinsichtlich der Anaesthesie und des Endoskops jedoch Vorschläge machen, die das Verfahren für Patienten und Operateur noch erleichtern. Diese Vorteile ergeben sich aus der Verwendung der Neuroleptanalgesie ohne zusätzliche Intubationsnarkose und eines nach dem Prinzip des Haslinger Rohres entwickelten Endoskops.

Die Neuroleptanalgesie wurde 1959 von de Castro und Mundeleer eingeführt und 1963 erstmals von Leicher in der Hals-Nasen-Ohrenheilkunde verwandt. Eine u W erste eingehende Beschreibung der Anwendung der Neuroleptanalgesie bei der Mikrolaryngoskopie haben wir bereits an andere Stelle gegeben (Eickhoff & Salehi, 1969)

Hervorstechendes Merkmal der Neuroleptanalgesie ist die getrennte Steuerbarkeit von Neuroleptikum und Analgetikum bei Erhaltung des Bewußtseins des Patienten. Nach Prämedikation mit Atropin verwandten wir Dehydrobenzperidol® und Fentanyl® in der durchschnittlichen Dosierung von 25 bzw 0 15 mg. Zur Verstärkung der Analgesie wurde zusätzlich eine Schleimhautanästhesie durchgeführt. Die für unsere Zwecke bedeutungsvollste Wirkung der Neuroleptanalgesie besteht in einem Mineralisationseffekt auf die Kehlkopfmuskulatur deren Starre den Eindruck einer Versteinerung vermittelt. Sie wird durch die erhaltene Spontanatmung nur unwesentlich beeinflußt. Dieser Zustand gestattet eine exakte Untersuchung mit Hilfe des Mikroskops bzw ein stimmschonendes mikrochirurgisches Vorgehen.

Neben der Ruhigstellung der Kehlkopfmuskulatur ist die Erhaltung des Bewußtseins der Durchführung der Mikrolaryngoskopie förderlich. Der Patient bleibt kooperativ das heißt, er kann jeder Aufforderung zur Bewegung der Kehlkopfmuskulatur nachkommen. Dadurch werden sogar intraoperative Funkt



Abb 1 In Anlehnung an das Haslinger Endoskop gebautes Laryngoskop. Konisches Rohr mit abgerundeter anatomiegerechter distaler Öffnung. In Verlängerung des Autoskopstutzens oben ein Pistolengriff nach unten.

gen der Stimme stroboskopische Untersuchungen sowie Tonbandaufzeichnungen ermöglicht.

Während der Neuroleptanalgesie bietet sich dem Untersucher ein übersichtliches, vom Intubationsschlauch freies Untersuchungs- bzw. rbeitsfeld, das den gesamten glottischen und bglottischen Raum umfaßt. Ein Lagewechsel Intubationskatheters aus der üblichen Interarytänoidstellung in die vordere Kommissur zur Freimachung der Arygegend und eine damit notwendige erneute Einführung des Endoskops erübrigen sich Auch kleine Kehlköpfe, die eine gleichzeitige Verwendung von Intubationsrohr und Endoskop nicht gestatten, ermöglichen unter der alleinigen Neuroleptanalgesie eine Mikrolaryngoskopie

Während der Neuroleptanalgesie können unter dem Operationsmikroskop die Untersuchungen und Eingriffe minutiös und ohne Zeitdruck vorgenommen werden, da die narkosespezifischen Belastungen für den Patienten nur gering sind. Die Kreislaufverhältnisse sind weitgehend stabil und jederzeit steuerbar Gefahr durch Aspiration von Speichel und Blut besteht wegen Erhaltung der Reflexe und evtl. zusätzlicher Tieflagerung des Oberkörpers nicht.

Außerdem lassen sich Blutungen durch Adstringentien und Saugkoagulation zuverlässig stillen. Darüberhinaus sind Operateur und Anaesthesist bei etwaigen bedrohlichen Blutungen, die wir jedoch nie erlebt haben, auf die Intubation mit Abblocken der Trachea stets vorbereitet.

Bei Beachtung entsprechender Vorsichtsmaßregeln kann die Mikrolaryngoskopie in Neuroleptanalgesie auch ambulant durchgeführt werden Grundsätzlich sollte aber eine Begleitperson für den Heimweg bereit stehen und der Patient auf die Führung eines Verkehrsmittels verzichten Erfahrungsgemäß sind bedrohliche Blutungen oder Gewebsschwellungen nicht zu erwarten Die Vorteile einer ambulanten Untersuchung ergeben sich u. a. aus der Bettennot und der Kostenersparnis für einen stationären Krankenhausaufenthalt.

Als Endoskop benutzen wir ein abgewandeltes Haslinger Rohr das sich wegen seines Pistolengriffes bei der Einstellung des Kehlkopfs durch seine besondere Führigkeit auszeichnet (Abb. 1)

Die Länge des Endoskops wurde entsprechend den topographischen Verhältnissen reduziert, die distale Öffnung der Konfiguration und Größe des Kehlkopfs angepaßt. Nach dem



Abb 2 Laryngoskop nach Kleinsasser Autoskopansatzstutzen gleichzeitig Handgriff.

Verlauf des Strahlenganges der Doppeloptik für die sterische Betrachtung wurde das Rohr konisch geformt.

Ungünstige anatomische Verhältnisse wie lange, vorstehende Zähne, dicke Zungen- und kompakte Nackenmuskulatur eine starre Halswirbelsäule sowie ein kaudal verlagerter Larynx lassen sich nach unseren Erfahrungen mit dem Haslinger Rohr leichter überwinden als mit dem üblichen Endoskop, wenngleich auch der Verwendung des Haslinger Rohres durch extreme Hindernisse Grenzen gesetzt sind. Bei Schwierigkeiten der Glottiseinstellung sollte man bei eingelegtem Rohr nach Empfehlungen Kleinsassers und eigenen Erfahrungen bis zu 10 Minuten warten, da nach dieser Zeit die Larynx-Muskulatur weiter erschlafft.

Bei den zur Zeit gebräuchlichen Endoskopen für die Mikrolaryngoskopie dient der nach oben stehende Ansatzstutzen für die Haltevorrichtung gleichzeitig als Handgriff zum Einführen des Rohres im Übergriff (Abb 2) Ein Laryngoskop jedoch nach den Prinzipien des Haslinger Rohres mit Pistolenhandgriff in Achsenverlängerung des Autoskopstutzens erleichtert die Einstellung des Larynx wegen

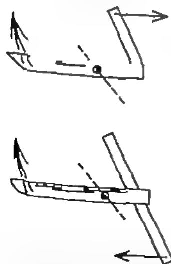


Abb 3 Skizzierung der Arbeitskräfte beim Einführen eines Laryngoskops. Oben: übliches Laryngoskop. Unten: modifiziertes Haslinger-Endoskop mit Pistolengriff. Einzeichnung der gedachten Drehachse etwa in Höhe der Zahnleiste.



Abb 4 Arbeitssituation bei Einführen des modifizierten Haslinger-Endoskops Hand im Untergriff, adduzierter Arm.

günstigerer Ausnützung der Armfunktion Die bewegenden Kräfte sind bei beiden Rohren zwar für Ober und Untergriff gleich groß aber entgegengesetzt gerichtet und können daher von der Hand nur in unterschiedlicher Art auf das Instrument übertragen werden (Abb 3) Beim Obergriff hat die Hand im wesentlichen eine Zugbewegung auszuführen, beim Untergriff einen Druck. Beim Pistolengriff arbeitet man mit adduziertem Arm (Abb. 4), beim Obergriff dagegen notwendigerweise mit abduziertem Arm, um sich den Blick in den Larynx durch das Endoskop nicht zu versperren (Abb 5)

Im einzelnen erklären anatomische Zusammenhänge die Leichtigkeit der Arbeit mit Pistolengriff bei adduziertem Arm (Ludwig)

1 Die optimale Ausgangsstellung für jede Bewegung im Handgelenk ist die Handkantenstellung in der Sagittalebene. Diese Orientierung ist beim Pistolengriff gegeben.

Abb. 1 In Anlehnung an das Haslinger Endoskop gebautes Laryngoskop. Konisches Rohr mit abgerundeter anatomiegerechter distaler Öffnung. In Verlängerung des Autoskopstutzens oben ein Pistolengriff nach unten

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Abb. Laryngoskop nach Kleinsasser. Autoskopstutzen gleichzeitig Handgriff.

VIBROTACTILE THRESHOLDS IN PURE TONE AUDIOMETRY

A. Boothroyd and S Cawkwell

From the University of Manchester Manchester England

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Research into vibrotactile sensations at the ear is emphasized by the problem of distinguishing them from auditory sensations. Normal hearing subjects may have difficulty in dissociating the two sensations while profoundly deaf subjects may never have experienced an auditory sensation. This paper describes experiments with unilaterally deaf subjects. It was found that they could consistently distinguish between the two sensations and could therefore give a measure of vibrotactile threshold. The results indicated that vibrotactile responses are very probable when testing profoundly deaf subjects with a standard clinical audiometer, and that individuals vary considerably in their vibrotactile sensitivity. It was not found possible to predict vibrotactile sensitivity at the ear from that at the fingertips.

It has long been known that when sound vibrations reach a sufficiently high intensity they may be perceived through the sense of touch. The relationship between this vibrotactile sensitivity and auditory sensitivity has been studied by a number of writers, but the implications of their findings in clinical audiology have not, in general, been widely recognized.

It is important in testing severely or profoundly deaf subjects to know at what levels one should expect responses to vibrotactile stimulation. To report vibrotactile air conduction thresholds may lead to the assumption of residual hearing when, in fact, none exists, while reporting similar thresholds in bone conduction testing may lead to the faulty diagnosis of a middle ear condition through the presence of an apparent air/bone gap

Based on "Vibrotactile Thresholds in pure Tone Audiometry" by S. Bayne, (now Cawkwell) Diploma Dissertation, Manchester University

In Fig. 1 are shown the thresholds of tactile sensitivity to air-borne sound ... have been reported by various workers. The data of Wegel (1922, 1932) and of Fletcher (1953) were obtained from normal listeners reporting "feeling" sensations as a result of high energy stimulation. It will be seen that these thresholds are generally higher than those given for deaf subjects. An early determination of vibrotactile thresholds of "deafmutes" was made by Schindler (1936) and the values found by him are very similar to those given by more recent workers (Groen, 1950; Langenbeck, 1965 Barr 1954 Nober 1967). The results which show the most disagreement with the general trend are those of Barr who indicates vibrotactile thresholds at levels of 100 dB for frequencies of 1 and 2 kHz. It should, however be pointed out that Barr's results show the lowest thresholds of 38 "totally deaf" children and Barr himself felt that these responses could equally well have been to vibrotactile or auditory stimulation.

In bone conduction audiometry the problem of vibrotactile stimulation becomes more acute. This is because the vibrator is specifically designed to transmit mechanical vibrations to the mastoid region. In consequence of this, the phenomenon has been more widely recognized in relationship to bone conduction testing. In a survey of the population of a school for the deaf Hughson *et al.* (1939) felt that the bone conduction thresholds obtained at 128 and 256 Hz were definitely the result

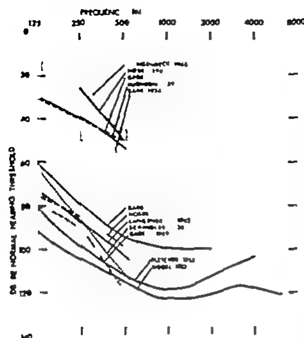


Fig 1 Published data for tactile responses to sound by bone conduction (*upper curves*) and by air conduction (*lower curves*). As f r as we know all the air conduction data were obtained using earphones. The data published with reference to physical zero has been corrected to the ISO (1964) threshold values. Data published in Europe prior to 1964 and expressed with reference to clinical zero has been left unchanged. All the bone conduction data are given as published. In iew of the difficulties of international standardization of bone conduction thresholds, it is probable that the reference levels vary from ter to writer

of vibrotactile stimulation although they were satisfied that the higher frequency thresholds were auditory Many writers have referred to this aspect of bone conduction audiometry without giving quantitative data For example Newby (1964) has said at 250 Hz the patient may respond to the vibrations of the oscillator without actually hearing the tone" (*Audiology* p. 81) Similarly Portmann & Portmann (1961) say "one must explain (to the patient) that with the low tones, for example, it is the threshold of auditory sensation which is being looked for (sonorous) and not the threshold of tactile sensation (vibratory)" (*Clinical Audiometry* p 11) More recently Reger (1965) has stated " patients with severe hearing losses for bone conducted sounds may respond to the vibrator with the vibration sense (pallesthesia) before the bone conduction threshold is stimulated, thereby giving a fake result" (*Audiometry Principles and Practices* ed. Glorig, Pure Tone Audiometry" p. 120).

Quantitative data on vibrotactile thresholds in bone conduction testing have been given by Groen (1950) Langenbeck (1965) and Barr (1954) and are illustrated in Fig. 1 Also shown in the figure are the results of Nober (1963 1964) who has carried out perhaps the most extensive work on this aspect of the problem

Several workers have referred to the possibility of other types of response to sound vibration. For example, Bocca & Perani (1960) have suggested that the bone conduction thresholds of severely deaf patients may represent "vestibular hearing" Similarly the feeling" thresholds for air borne sounds found by Wegel (1932) were reported by his subjects to produce sensations which could be related to vestibular disturbance, and Schindler (1936) quotes threshold results for influence of the vestibular apparatus" which are different from those produced by vibrotactile stimulation of the ear Nober (1964) does not accept the theory of vestibular hearing, however one of his strongest arguments being based on the similar configuration of bone conduction thresholds of profoundly deaf subjects and their vibrotactile thresholds at the fingertips

While many educators may have acclaimed the discovery of residual hearing" in a large percentage of the deaf population without a critical examination of the possible interpretations of the experimental evidence others have recognized the possibility that much so-called residual hearing may be tactile Several, however have proceeded to use vibrotactile sensitivity as a possible channel of communication. For example van Uden (1958) has stated,

all deaf children can react to sound in the ears in a certain percentage of our deaf children these reactions seem to be very similar to vibration feeling. It seems to be probable that these children have only vibration feeling

in the ears. He has, however, developed educational techniques to make maximum use of this sensitivity.

Emphasis on the vibrotactile sense has also been placed by Guberina (1963) who has transmitted information through vibrators placed on various parts of the body. He has stated "... it is not by pure chance that a deaf person remains sensitive to the low frequencies which are commonly called 'vibrations'." It is not clear why Guberina should put vibrations in quotes. The point in question is not the physical nature of the stimulus, which is undoubtedly vibratory, but of the sensory modality through which it is perceived. Possibly Guberina feels it unnecessary to make a distinction between the auditory and tactile sensations and wishes to emphasize the communication value of the tactile sense.

The relevance of vibrotactile sensitivity to the fitting of hearing aids has been discussed by Bellefleur & Smith (1967). Again, these writers have made the point that perception through the tactile sense does not necessarily imply the inability of deaf people to benefit from amplified sound.

In examining the thresholds quoted in the literature for responses to vibrotactile stimulation, two points should be borne in mind. Firstly normal hearing subjects must experience a simultaneous auditory sensation when exposed to sound intensities sufficient to produce vibrotactile stimulation within the usual frequency range of pure tone audiometry. Under these conditions, the two are perceived as a whole and unless the observer is able to dissociate the sensations he may be unaware of the vibrotactile element until it is well above threshold levels. This may account for the higher thresholds found by Wegel and Fletcher. Secondly, when using profoundly deaf subjects it is difficult to decide whether they are responding to an auditory or a vibrotactile sensation, since a person who is sufficiently deaf as to have no experience of auditory sensations will be unable to make the subjective distinction. Those with true residual hearing may be able to do this, however. Barr (1954) quotes the case of a deaf boy who, after many years of testing, informed the tester that his responses to air conduction testing were to an auditory sensation while his responses to bone conduction testing were to a tactile sensation. Because of their inability to give a reliable subjective report as to the nature of the sensation it has generally been necessary, in interpreting the results of tests on profoundly deaf subjects, to infer this from such features as air bone gaps in populations with no middle ear disorders, the similarity of threshold configurations with those obtained from vibrotactile stimulation of non-auditory areas, the uniformity of threshold curves among populations having various causes of deafness, and the differential reaction to masking of the auditory and vibrotactile thresholds (Nober 1963–1964 and 1967). Recent work by Risberg (1968) however, indicates that it may be possible to reliably distinguish auditory from tactile responses in terms of the discrimination of periodic and aperiodic sounds.

It has apparently been overlooked that a ready source of experimental material exists in the form of subjects with profound unilateral deafness. Such subjects have virtually normal auditory perception, but at the same time, it is possible to investigate their responses to high intensity stimulation of the deaf ear. The present paper describes the results of a pilot study carried out on unilaterally deaf subjects and although the sample was small, it was felt that the results were sufficiently definitive to warrant publication at this stage.

Aims of the Experiment

The aims of the experiment were as follows:

(i) to determine whether unilaterally deaf subjects could reliably distinguish between vibrotactile and auditory sensations at the deaf ear;

(ii) to determine the range of vibrotactile threshold responses to be expected in air conduction and bone conduction testing;

(iii) to see whether vibrotactile thresholds of a region remote from the ear could be used in predicting thresholds at the ear

Subjects

Nine subjects aged between 10 and 15 years were used in the experiments. Each had previously been diagnosed as having a profound unilateral deafness with normal or near normal hearing in the better ear Seven of the subjects were boys and two were girls. Choice of age range and individual subjects within this range were determined by accessibility in a school population close to the University of Manchester the setting for this research.

EQUIPMENT

A Peters Audiometer Type AP6 were used for all threshold measurements. This was fitted with a 20 dB booster to permit air conduction testing at levels above those normally available on clinical audiometers. Thresholds for vibrotactile stimulation of the fingertips were determined with the bone conduction vibrator of the audiometer the reference level being that of the auditory thresholds for mastoid placement in normal hearing subjects. Pressure at the fingertips was applied by a device designed earlier research by Wilson (1967), which arranged to apply a force of 300 g weight. This is approximately equal to the force applied by the headband in normal bone conduction testing.

PROCEDURE

The frequencies used for threshold determinations were 250, 500 750, 1 000 and 2,000 Hz. Under all test conditions. it was necessary to mask the better ear of the test subject. That this should be necessary in both air and bone conduction testing is obvious, but even when measuring thresholds at the fingertips sufficient sound was radiated from the vibrator and heard by the subject through the better ear to make the distinction between auditory and tactile sensations extremely difficult. In the bone conduction tests, measurements were also made at 4 000 Hz, but no responses were obtained at the maximum level available. The required masking levels were too high to permit air conduction tests at this frequency Narrow band masking was used throughout and the shadow technique of Hood (1960) was employed. This involved determining threshold levels for increasing levels of masking, it being assumed that sufficient masking was employed when the measured threshold was independent of masking level for a range of 20 dB of masking. Throughout the testing, the subject was questioned as to the nature of the sensation to which he was responding, and he was instructed to respond regardless of the nature of this sensation Care was taken not to influence his answers by such questions as "Can you feel it yet?" but rather "Tell me whether you hear it or feel it"

RESULTS

Fig. 2 shows the median values and ranges of vibrotactile thresholds determined in the experiment. All of the subjects were able without difficulty to distinguish between auditory and tactile sensations. At the fingertips and at the mastoid, all nine subjects reported that the threshold levels finally determined were of a tactile sensation. However one of the nine subjects reported that the masked air conduction threshold was that of an auditory sensation. The threshold levels of this ninth subject are shown in Fig. 3 and it will be seen that the air conduction threshold was well outside the range of vibrotactile thresholds obtained from the other eight subjects.

No evidence was found of a positive correlation between sensitivity at the ear and at the fingertips.

DISCUSSION

A striking feature of the results is the wide range of vibrotactile thresholds, particularly at

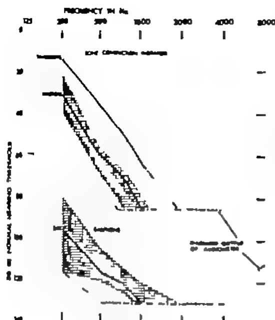


Fig. 2. Heavy lines show the median values of vibrotactile thresholds found in the experiments. The ranges of threshold values are indicated by the shaded areas.

1 and 2 kHz most probably indicate the presence of true residual hearing.

It is important to examine the vibrotactile thresholds in relation to the maximum output of standard clinical audiometers. Without the 20 dB booster available on the machine used in this experiment, air conduction thresholds would have been measurable only at frequencies of 500 Hz and below and hen only for two or three subjects Similarly an audiometer whose bone conduction output is limited to 65 dB would not have been able to produce vibrotactile stimulation of the mastoid area in any of the nine subjects at frequencies above 500 Hz. However even with a standard clinical audiometer there is a possibility that reported thresholds will be those of a vibrotactile sensation in a certain percentage of profoundly deaf subjects for frequencies of 500 Hz and below

the fingertips and for low frequency earphone stimulation. Had more consistent thresholds been found, it might have been possible to indicate a line on the audiogram form below which vibrotactile sensations would almost certainly be experienced by a test subject. However, one can say that within the shaded areas shown in Fig. 3 there is a strong possibility of the occurrence of vibrotactile stimulation, and that a deaf subject whose air and bone conduction thresholds fall within or below these areas might well be totally deaf.

It was hypothesized that the sensitivity at the fingertips of an individual subject might be used to predict his vibrotactile sensitivity at the ear However no correlation was found and such prediction does not appear possible

It will be seen from Figs. 1 and 2 that the vibrotactile thresholds found in this experiment are similar to those found by other researchers, the most noticeable exceptions being the high frequency air conduction thresholds given by Barr On the basis of the present findings one would assume that thresholds of 100 dB at

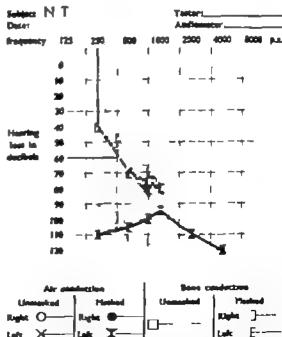


Fig 3 Threshold responses of the ninth subject to stimulation of the 'deaf' ear His subjective report was that the bone conduction thresholds were to vibrotactile stimulation while the air conduction thresholds were to auditory stimulation. A comparison with the thresholds of the other eight subjects is given.

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DIRECTIONAL AUDIOMETRY

I Directional White-noise Audiometry

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From the State Hearing Center Aarhus Denmark

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The construction and calibration of a device for the angular localization of white noise is described. The apparatus has been used to measure directional hearing abilities in the horizontal plane in 30 normally hearing subjects using white noise. A standard graph of the results of the tests has been prepared, showing the limits within which 95 per cent of the localizations were made. This diagram will serve as a basis for comparison when examining the angular localization of noise in patients with hearing loss.

In order to measure a person's ability to localize the direction of a noise source in the horizontal plane, a noise source which can be randomly placed on a circle in that plane is uired. The person to be examined is at the center of this circle, with the ears the reference plane. With this form of free field audiometry it is also possible to examine people with hearing aids. One of the primary reasons for the construction of the device to be described has been to analyse the influence of modern hearing aids on the patients' abilities to localize the direction of white noise sources. To have a basis of comparison, the directional hearing of 30 normally-hearing persons has been examined.

Significant Factors in Directional Hearing

Directional hearing in normal, binaurally-hearing subjects is due mainly to the following factors.

1 *Differences in intensity* The ability to localize sounds is dependent upon the difference in the intensity of the sound striking the right and left ear (Stewart & Hovda, 1918) Experimental evidence seems to indicate that this difference in intensity is important especially at frequencies over 300 Hz. (Sivian & White, 1933 Rosenberg & Slavinsky 1940 Nordlund & Lidén, 1963)

2. *Differences in phase* The ability to localize sounds is dependent upon phase differences in the sound impulses striking the right and left ear (Firestone 1930; Wightman & Firestone 1930) Jongkees & Groen (1946) believe that this phase difference is significant only by localizing sounds with frequencies under 1000 Hz. Sandel *et al.* (1955) found the limit as high as 1500 Hz., while Kietz (1957) as well as Christian & Röser (1957) state that the limiting frequency is about 800 Hz.

3 *Differences in time* The ability to localize sounds is dependent upon differences in time of arrival of sound at the right and left ears (Békésy 1930 Wallach *et al* 1949 Kietz, 1957 Matzker & Springborn, 1958). The difference in time of arrival is especially important by the localization of complex sounds (Christian & Röser 1957) In the localization of such a source they found no difference if the sound was composed primarily of high frequency or low frequency tones.

4 *Tone colour* The ability to localize complex sounds is, furthermore dependent upon the fact that the tone colour changes with the

angle of incidence (Angell & Fite, 1901 Steinberg & Snow 1934)

5 *Frequency* The ability to localize pure tones varies with the frequency It is approximately constant below 1000 cycles, drops rapidly to a minimum between 2000 and 4000 cycles, and rises again to its former level at higher frequencies Stevens & Newman (1936).

6. *The presence of the auricles* also plays a role in the directional perception of sound, which has been mentioned in several papers (Batteau, 1967 1968 Fisher & Freedman, 1968)

7 *Movements of the head* In making a physiological determination of the direction of a source of sound, it is important that the head is not fixed. Movements of the head increase the ability to localize noise sources (di Carlo & Brown, 1961) Voluntary and reflex movements of the head have been the subject of experimental research (Wallach, 1939 Klensch, 1948 Jongkees & van der Veer 1958)

The Selection of White Noise as Stimulus

Having considered the factors listed above, white noise was selected as stimulus in this directional audiometric examinations. The measurement of directional bearing by use of pure tones would require testing a whole series of frequencies. This is impractical when testing the patients. Most of the sounds one meets in daily life are complex, and thus have significant characteristics in common with white noise, in respect to the localization of sound sources. A noise level of 65 dB is an everyday occurrence. (Whenever decibel, dB is mentioned in this article, the reference level is 0.0002 dyne per square centimeter if nothing else is specially mentioned.) The white noise used here had an intensity of 65 dB at the center of the orbital path of the loudspeaker The person examined was placed so that the midpoint between the ears corresponded to this center The intensity was measured without a subject in the test area.



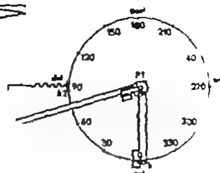




Fig 1 Arrangement of the equipment in the anechoic room.
wn. Brüel & Kjær Random Noise Generator Type 1402.
tr Telefunken Magnetophon Type 85 Scotch Magnetic Tape, 19 cm/sec.
c Change-over switch.
i. Interrupter switch.
at: Radiometer attenuator type Lp 7b.
a. Newcord amplifier type TFS 76.
k1 Loudspeaker Peerless type E32 MT cone diameter 7.5 cm, mounted in 1.2 liter pressure baffle
k2. Loudspeaker Philips type AD 346 X, mounted in 2.1 liter pressure baffle.
and Noise generator in an Amplex Audiometer type 0B1
p. Position gear for loudspeaker k1
m. Motor for loud speaker arm.
k. Rockwool wedges.
P1 Person examined.
P2. Operator

Apparatus

Not all of the functions of the apparatus to be described here were used in the examinations published in this paper Other functions are, however used in experiments which are to be published later It is therefore rational to describe the entire apparatus in this publication.

The equipment, the observer and the person being examined were placed in an anechoic room (Fig. 1). The dimensions of the room were 4.75 meters long, 2.90 meters wide and 2.25 meters high. A loudspeaker (*h1*) could be moved on a circumference of a circle with radius 0.9 m centered corresponding to the center of the head of the person tested. This circle lay in a horizontal plane going through the ears of the seated subject to be examined (P1). In front of the investigator (P2) there was a selector (*p*) which permitted positioning of the loudspeaker (*h1*) at intervals of 30 degrees around the circle. Another loudspeaker (*h2*) could likewise be moved around the same center as *h1* in a concentric circle with radius of 0.95 m. The two speakers were so arranged that they could not shield one another. Speaker *h1* could reproduce either white noise from a noise generator (*wn*) or speech from a tape recorder (*tr*) as selected with the switch (o). Speaker *h2* could reproduce only white noise from a generator in an audiometer (*aud*). In addition, there was an interrupter (*I*), an attenuator (*at*) and an amplifier (*a*). In the experiments reported in this paper only speaker *h1* was used. Both speakers were hidden from the subject by a thin but nontransparent black ...tton curtain. On the side facing the subject ...curtain was marked at intervals of 2.5 de-...s with numerals and letters, which were used by the subject to indicate the apparent position of the noise source, i.e., where the speaker was thought to be. The subject to be examined was sitting in an arm chair with a head rest, allowing movements of the head.

Calibration of the Apparatus

In making the intensity measurements described below in paragraphs, 1, 2, 4 and 5 a Brüel & Kjær Sound Level Meter Type 2203 and Microphone Type 4131 were used.

1. With white noise produced by one speaker at a time, measurements showed that the sound level dropped approximately 6 dB when the distance from the speaker was doubled (from 0.45 to 0.9 m) indicating that the room was sufficiently anechoic for our purposes.

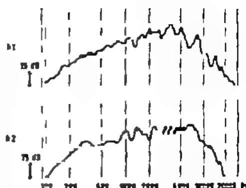


Fig. 2. The frequency response of the loudspeakers h1 and h2.

2. Placing the microphone at the center of the orbital paths of the speakers, and using white noise at the level of 65 dB, the speakers were turned ±10° in the horizontal and vertical planes from their axes in the speaker mountings while the sound level was measured continuously. No measurable variation in the sound level was recorded, indicating that the radiation pattern of the speakers was essentially spherical in the zone where the head of the person examined was to be located.

3. With a Brüel & Kjær Automatic Frequency Response Recorder Type 3308, Microphone Type 4131 curves were prepared for each speaker (Fig. 2).

4. At the point serving as the common centre of the subject's head and the concentric paths of the speakers, the noise level at the 65 dB setting was measured with speaker *h1* in each of the 12 positions. Only in the 120° and 210° positions could a variation be observed with certainty (−0.5 dB and −0.6 dB respectively). These variations were ignored.

5. Corresponding measurements of the speaker *h2* in its four positions did not indicate any significant measurable variation.

TEST MATERIAL

Persons with normal hearing were used as test subjects. They were considered to have normal hearing when the threshold for air-conducted

Table 1

Loudspeaker position ...	0	30°	60°	90	120°	150°	180°	210	240	270	300	330°
M	−0.17	+1.42	0.50	+0.33	+0.50	−0.75	−0.33	1.33	−1.08	−0.17	−0.58	−1.58
s_x	3.33	3.18	3.36	3.51	4.21	4.82	4.62	4.57	4.23	3.58	3.18	3.90
$\pm s_x t$	6.81	6.50	6.87	7.18	8.61	9.86	9.45	9.35	8.65	7.32	6.50	7.95
Upper limit	+6.64	+7.92	+7.37	+7.51	9.11	9.11	+9.12	+10.68	+7.57	7.15	5.92	6.40
Lower limit	−6.98	−5.08	−6.37	−6.85	−8.11	−10.61	−9.78	−8.02	−9.73	−7.49	−7.08	−9.56

Results of the measurements of directional hearing made on 30 experimental subjects with normal hearing. The figures in the table give the deviation in degrees from the actual position of the loudspeaker clockwise deviation ( ), counterclockwise deviation (−).
M. Arithmetical mean.
s_x. Standard deviation. (Variance corrected according to Sheppard.)
$s_x t$. Indicates in degrees the distance from M to the limits of 95 % significance ($t = 2.045$).
Upper and lower limits: The limits are calculated to include 95 of the population around the actual position of the noise source.

tones in the range from 125 to 8000 Hz was 10 dB hearing loss or better (British Standard), and in the range from 500 to 2000 Hz, no hearing loss (threshold 0 dB hearing loss or better) was required. Frequencies tested. 125 500, 1000, 2000 4000 6000 and 8000 Hz. This preliminary audiometry was performed with a Madsen Electronics Audiometer Type OB 60, calibrated according to British Standards 1954 while reference is also made to Whittle & Robinson (1961)

The test subjects were 16 women and 14 men, from 19 to 52 years old, 17 of them between 19 and 24 years old. All were right handed, and had negative histories of diseases of the ear and central nervous system. By clinical examinations the outer ears, the meatus and the ear drums were found to be normal.

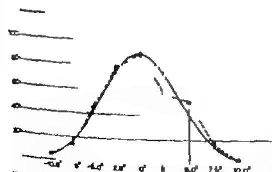


Fig. 3. The distribution of the 360 observed results (- - -) compared with the normal distribution (——). *Abscissa*: deviation in degrees of the subjective position of the loudspeaker from the objective position. Positive numbers indicate clockwise deviation; negative numbers indicate counterclockwise deviation. *Ordinate*: N = numbers of observations. The observed data are in agreement with the normal distribution ($\chi^2 = 6.91 < P_{.05}$, 4 degrees of freedom).

METHOD

During the examination of each person, the speaker was placed in the 12 different positions according to a table of random numbers. While the white noise signal was presented for a period of approx. 10 sec, the subject was permitted to turn the head about 5 to the right and left. When the noise was switched off the subject was requested to indicate the direction of the noise source. If it was thought that the noise came from behind, the subject was asked to turn and read off the symbols indicating the direction of the noise source.

RESULTS

The results of the examinations of the 30 subjects are presented in Fig. 3 where the distribution of the 360 recorded results are indicated. We assume the position indications to be normally distributed. (Statistics in this article: According to Dixon & Massey 1957.)

The figures in Table 1 are for each position of the loudspeaker giving the degrees of deviation of the indicated positions from the actual positions. Positive numbers indicate clockwise

deviation, negative indicate counterclockwise deviation. The mean (M) and the corrected standard deviation (s) for each position are calculated, together with the limits inside which 95% of the localizations were made. Because of a class interval of 2.5 degrees Sheppard's correction was used, subtracting $^{1}/_{12}$ of the interval from the variance.

To permit a clear visualization of the test results, the results are graphically presented in Fig. 4 in a coordinate system similar to that used by *Jongkees & Groen (1946)*.

In Fig. 4 shows the objective position of the source (*abscissa*) and the subjective position (*ordinate*). Correct localization of the sound source will thus result in points lying on the line shown in the second and fourth quadrants of Fig. 4 and forming an angle of 45° with the axes. Incorrect localizations will thus result in larger or smaller deviations from this line.

On the basis of the figures calculated in Table 1 two additional dotted lines have been drawn on Fig. 4 giving the approximate limits of the area covering 95% of the results of the directional hearing tests made on the 30 normally-hearing subjects examined. As our experiment permits indication of the location of a sound source with a fineness of 2.5 degrees at best, practical use of the graph has necessitated rounding up the calculated 95% probability limits to correspond to the graduation on our apparatus. These approximated limits to the 95% probability band have been drawn in Fig. 4 (dotted lines).

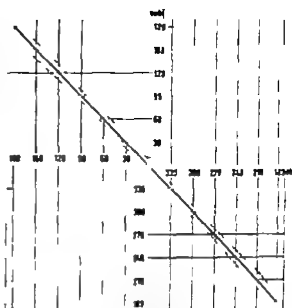


Fig. 4. Directional hearing ability of 30 persons with normal hearing. White noise is used as stimulus. Graphical presentation of the objective position (*abscissa*), and the corresponding subjective position (*ordinate*) of the sound source. Solid line represents the ideal, correct localization. The area between the dotted lines on each side of the solid line covers approximately 95% of the results.

COMMENTS

Jongkees & Groen (1946) and van der Veer (1957) made use of a similar coordinate system. They investigated directional hearing of pure tones in a horizontal plane on that part of a circle lying in front of the subject (the half circle from 270° to 0° to 90°). They found that a normally-hearing subjects deter minations, when plotted in their coordinate system, resulted in an S shaped curve. The shape of this curve was statistically verified by van der Veer (1957). If we examine our results in Table 1 it is possible to discern the same tendency. The band giving the approximate limits of the zone containing 95% of the indications shows a twisted path, also in the area behind the subject (from 90° to 180° to

Table 2. *Variance of the deviation of the mean by localizing the noise source. Analysed for loudspeaker positions and for persons tested.*

F_{posit} shows a significant difference between the positions. F_{pers} shows a highly significant difference between the persons.

Variation	Degrees of freedom	Sum squares	Mean squares
Between positions	11	43.7639	3.9785
Between persons	29	211.1139	7.2798
Remainder	319	671.9861	2.1065
Total	359	926.8639	[illegible].5818

$F_{posit} = \frac{3.9785}{2.1065} = 1.889 \qquad F_{posit} > P_{.05}$

$F_{pers} = \frac{7.2798}{2.1065} = 3.456 \qquad F_{pers} > P_{.001}$

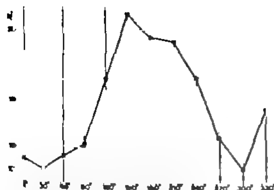


Fig. 5 Coordinate system, illustrating the accuracy in directional hearing expressed as s_e (*ordinate*) as a function of the azimuth of the noise source (*abscissa*).

270). However analyzing our calculated means, we find that M does not differ significantly from ▮ except for the positions 30 and 330 (Test used: 0-hypothesis rejected if 0 is outside the limits of

$$M \pm t \frac{s_e}{\sqrt{n}}, t > P_{.95})$$

However both for the different persons and for the different positions of the noise source, analysis of the variance of the deviation of the mean shows a marked significance (Table 2)

Each of the persons tested made a systematic error of a personal amount by indicating the subjective position of the noise source For each of the objective positions of the noise source, we found a systematic error by indicating the subjective positions of the source

The accuracy in directional hearing ability appears to be a function of azimuth of the noise Expressed in the variance found in each position, this variation is at maximum between 150 and 210 and at lowest at 30 and 330 (Fig. 5)

However Bartlett's test for homogeneity of variances shows $\chi^2 = 14\ 005$ for 11 degrees of freedom, giving $\chi^2 < P_{.95}$. The conclusion is, therefore, that for our 30 experimental persons there is no significance in these variations.

Cochran's test of homogenity of variances shows no significant differences between the different persons in the accuracy to localize the noise source

The graph plotted in Fig. 4 gives an impression of the abilities of 30 persons with normal hearing to localize a white noise source This graph will be used as a basis of comparison during later tests, when examining and evaluating the localization of white noise by patients having impaired hearing, with and without hearing aids.

ZUSAMMENFASSUNG

Es wird die Konstruktion und die Eichung einer Anordnung für die winkel eise Lokalisierung weissen Rauschens beschrieben. Das Gerät ist zum Messen gerichteter Hörfähigkeiten im Horizontalplan bei 30 Versuchspersonen mit normalen Gehö unter Anwendung weissen Rauschens benutzt worden. Ein Standard-Diagramm der Versuchsresultate, das die Grenzen zeigt, innerhalb deren 95% der Lokalisationen gemacht wurden, ist angefertigt worden. Dieses Diagramm kan bei der Untersuchung winkelweiser Lokalisierung von Geräuschen bei Patienten mit Gehörschaden dienen.

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LATERALIZATION OF BONE CONDUCTION INTO THE BETTER EAR IN CONDUCTIVE DEAFNESS

Paradoxical Weber Test in Unilaterally Operated Otosclerosis

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Patients with bilateral asymmetric conductive deafness due to otosclerosis were investigated to establish how B.C. lateralization (Weber) is affected when the poorer ear is transformed by stapes surgery into the better ear B.C. lateralization was measured in 90 patients at different frequencies on the day before surgery and at various times up to maximum of 4 years post-operatively

Three types of post-operative behaviour of B.C. lateralization have been observed.

1 Simultaneous reversal—lateralization reverses to the contralateral ear as soon as the operated ear becomes superior to the non-operated one.

2. Late reversal—lateralization shifts into the contralateral ear some weeks or months after the operated side has become the better ear

3 No reversal—lateralization remains directed to the operated ear in spite of the fact that this side now has the better hearing. There is *Paradoxical Weber* lateralization int *better* ear in bilateral conductive hearing loss.

The factors ruling B.C. lateralization are discussed. The possible roles played by intercochlear intensity and intercochlear phase difference are analyzed and the presence of third factor, that of central habituation is suggested.

Since longstanding deafness produces relative sensory deprivation it is conceivable that sudden hearing improvement can cause temporary imbalance of the central evaluation of stimuli arriving from both cochleae. This may account for the phenomenon of gradual and late reversal of lateralization.

Bone conduction is lateralized into the better ear in sensory-neural deafness and into the poorer ear in conductive deafness. This phenomenon is one of the fundamentals upon which classical otological diagnosis was based. It is usually tested by means of a tuning-fork placed on the midline of the skull. This tuning-fork test is generally called the Weber test, although it appears that Weber did not describe any test of this kind. He described (1834) only the phenomenon of laterallization of bone conduction on occlusion of the external ear canal The first description of bone conduction lateralization as a diagnostic tool to distinguish between perceptive and conductive deafness was given by Schmalz (1846), as far as we have been able to determine (Huizing, 1967)

Explanation of B C Lateralization into the Conductive Impaired Ear

A satisfactory explanation of the phenomenon of lateralization in the bad ear in conductive deafness is still lacking. The old theory of the impaired "Schallabfluss (Mach) and the Masking Theory are both abandoned. According to a more recent hypothesis (Tarab 1958 Langenbeck, 1958) this lateralization results from a phase difference between the two cochleae, the ear with the conductive loss being leading in phase. In the experimental animal a change in the middle ear system indeed results in a cochlear phase shift (Legouix & Tarab, 1959)

From psycho-acoustic experiments it is known that in air conduction a pure tone stimulus, when offered to both ears with an interaural phase difference, gives rise to a l zation of the sound image into the ear,

is leading in phase. This holds especially for the lower frequencies (Garner & Wertheimer 1951)

In a previous personal study (Huizing, 1963) it was found that in air conduction for a 500 Hz tone lateralization is perceived at interaural phase differences from 40 to 140 with a maximum lateralization at 90 phase difference The intensity of this lateralization, however appeared to be rather weak, ranging from 50 % at a 40 phase difference to a maximum of 75 % at a 90 phase difference Moreover it was found that the maximal lateralization at 90 phase difference is already cancelled at an interaural intensity difference of 5 dB or more Almost identical data have been obtained by Naunton & Elpern (1964) in a similar experiment.

These and other experimental data (Sedee 1957 Groen, 1962) indicate that interaural phase differences form a factor that can contribute to the lateralization of bone conduction into the poorer ear in conductive deafness. It does not however seem to act as the only component in the generation of the phenomenon, which is still not well explained.

Present Study

be aim of the present study was to investigate w in cases of bilateral asymmetric conduc deafness, bone conduction (B C) lateralization is affected when the poorer ear has been transformed by stapes surgery into the better ear For this purpose lateralization was tested in 90 patients with bilateral asymmetric conductive deafness due to otosclerosis, before and after stapedectomy in the poorer ear In all these cases the stapes was totally removed and replaced by a gelfoam-wire prosthesis or a Teflon piston.

Included in this study were only those otosclerosis patients

(*a*) who showed a purely conductive impairment without sensory-neural loss, and

(*b*) in whom the operation had been successful and had led to a transformation of the originally poorer ear into the better ear

A preliminary report on the results of this study appeared some years ago (Huizing 1967)

TEST PROCEDURE

Weber-lateralization was determined pre and post-operatively by means of a B C. oscillator placed on the midline of the forehead. This site of application of the stimulus was chosen as it is the most commonly used in audiometrical Weber testing. The examination was carried out at 250 500, 2 000 and 4 000 Hz. All test frequencies were offered 10 times in a random procedure The intensity of the stimuli was 40 dB HL, the duration 1 sec. The patient had to chose between a right, left, and midline localization of the tone as is usual in the classical Weber test. The test was performed the day before surgery and post-operatively after 4 days, 11 days, 3 to 4 weeks, 2 months and thereafter according to the results. Pure tone audiograms were made at the same time, except during the first weeks after surgery

RESULTS

In the cases discussed here three different types of postoperative behaviour of bone conduction lateralization were observed.

1 *Simultaneous Reversal of Lateralization*

In the greater part (37) of the patients the lateralization appeared to shift into the contralateral ear as soon as the hearing of the operated ear became superior to that of the non-operated one This we call a simultaneous reversal" of the lateralization as it occurs approximately simultaneously with the transformation of the operated ear from the side with the greater conductive loss into the side with the smaller conductive loss. This reversal of lateralization is in accordance with our expectation, since we customarily find the Weber lateralization directed to the poorer ear in bilateral conductive deafness. In general, the reversal of lateralization was found to take

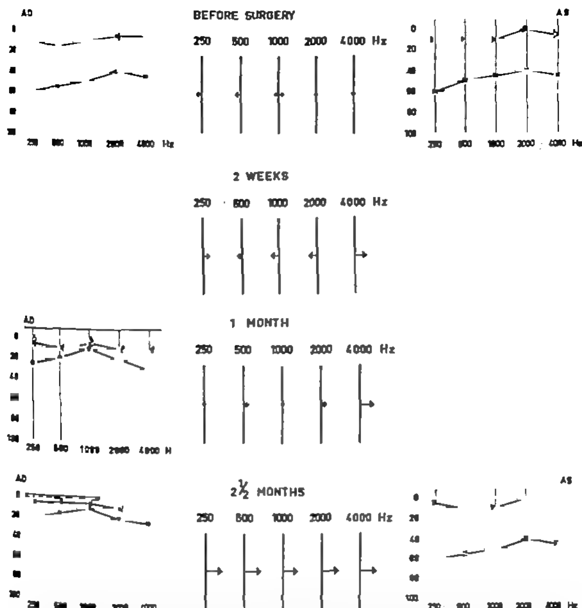


Fig 1 Late reversal of lateralization. Although the right ear has become the better hearing side 1 month after stapes surgery lateralization has not yet shifted into the now poorer left ear However 2 ½ months after surgery lateralization has completely reversed to the left

place within 1 to 6 weeks after surgery In most patients it took place for all frequencies at approximately the same moment, as the hearing improvement occurred.

2. *Late Reversal of Lateralization*

In a number of cases (24) however the lateralization did not shift into the contralateral ear simultaneously with the hearing improvement. It took weeks or months after the time at which the operated side had become the better ear before lateralization was reversed into the contralateral side. This we call a "late reversal of lateralization" Fig. 1 gives an example of this type

Frequently such a late reversal was found take place gradually In Fig. 2 a case is

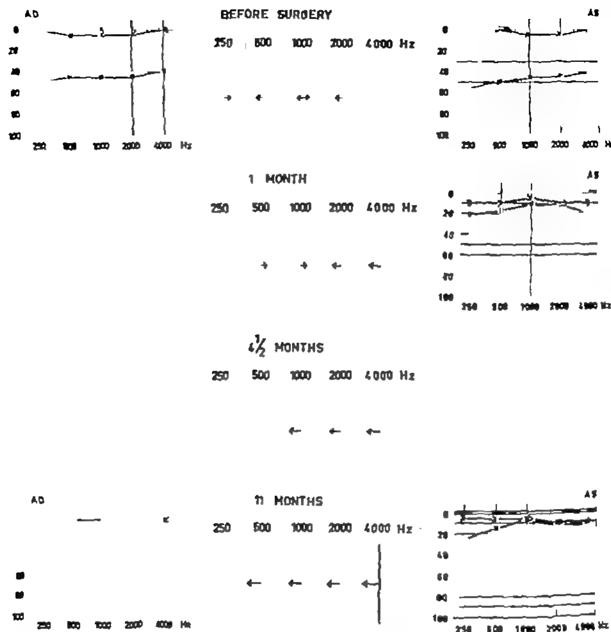


Fig 2 Gradual late reversal of lateralization. Gradual reversal of B.C. lateralization into the opposite ear in a period of several months after attainment of the definite hearing gain.

with a slow shift of the lateralization towards the opposite side which took place in a period of several months after the definite hearing improvement had been attained.

3 *No Reversal of Lateralization*

In 29 patients, B C lateralization did not shift to the opposite ear during a follow-up period of 1 to 4 years. It remained directed into the operated side i.e. into the ear with post-operatively the smallest amount of conductive impairment. The Weber test in these cases has a paradoxical outcome: *lateralization into the better ear in asymmetrical conductive deafness.* Two examples are given in Figs. 3 and 4 When comparing the A C. and B C thresholds of both ears we would expect in both cases a lateralization of B C to the ear with the

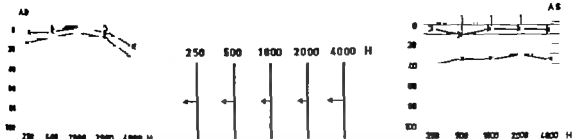


Fig. 3. Paradoxical Weber: B.C. lateralization to the better ear in conductive deafness 4 months after stapedectomy on the right ear. Lateralization was also to the right ear, being before surgery the side with the poorer hearing. Lateralization continued to be directed to this side after the right ear had become the better ear. The paradoxical Weber remained constant during the complete follow-up period of 20 months.

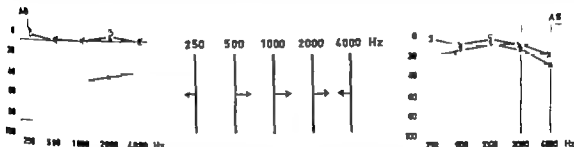


Fig. 4. Paradoxical Weber at 500, 1,000 and 2,000 Hz, 7 months after stapedectomy on the left ear. Before the operation, lateralization was also to the left ear being the side with the greater conductive impairment. For 250 Hz the Weber shifted to the right side 2 months post-operatively. For 4,000 Hz it reversed immediately probably as a result of small traumatic sensory-neural loss for the highest frequencies.

greater conductive loss. This appears not to be the case, however. In both these patients lateralization was found to be directed to the better (subnormal) ear during the complete follow-up period of 20 and 7 months, respectively.

In some patients the phenomenon of the paradoxical Weber was found for all test frequencies (Fig. 3), in others it was observed for some frequencies only (Fig. 4). Sometimes, the paradoxical Weber appeared to be a temporary phenomenon and a very late reversal was observed afterwards. In other patients the phenomenon remained present for the whole follow-up period ranging from 1 to 4 years.

DISCUSSION

Why a simultaneous reversal of the lateralization takes place in some patients whereas in others a late reversal or no reversal occurs is not easily explained.

Three mechanisms have to be considered as factors governing B.C. lateralization after stapes surgery in otosclerosis.

1. *Intercochlear Intensity Difference*

The first factor involved is the intensity difference between the two cochleae in bone conduction. It is well known that stapes surgery may influence bone conduction in otosclerosis in two different ways. In the first place, some high tone loss may result due to inner ear trauma. Secondly and more important for this discussion, is the bone conduction gain frequently observed in the middle frequency region and which is generally considered as the abolition of a "middle ear bone conduction loss" (also called Carhart notch).

From the experimental results we know that in air conduction an intercochlear intensity difference of more than 5 dB evokes lateralization in spite of an opposing phase difference. Therefore, a post-operative bone conduction improvement of some 5 to 10 dB (as is so frequently encountered for the middle frequencies) will probably maintain the lateralization in the operated ear irrespective of any intercochlear phase difference.

Intensity difference therefore plays an important role in determining lateralization. Although probably more important than phase difference, this factor too seems to be not completely dominant, as may appear from the following:

(*a*) It is generally found that a B.C. gain after stapes surgery (when present) mainly concerns the middle frequency region. So if intensity difference were the dominant factor we might expect a different behaviour of the lateralization for the various frequencies. This appears to be not the case: although the intensity of the lateralization often differs for the various frequencies, its direction is generally to the same side. This holds especially for the low and middle frequencies.

(*b*) The gradual reversal of lateralization observed in a period of months after the definite hearing improvement has been attained, also cannot be explained by an intercochlear intensity difference. In general, no further improvement of air or bone conduction can be expected later than three months after operation.

2. *Intercochlear Phase Difference*

A second factor to be considered is intercochlear phase difference. Replacement of a fixed stapes by a mobile prosthesis will change the mass and the stiffness of the middle ear system, which leads to a shift of the relative phase of the vibration of the cochlear contents. This phase shift will increase or decrease an existing intercochlear phase difference or in cases of symmetrical pathology produce such a difference.

As we know from the experiments mentioned above an interaural phase difference can evoke B.C. lateralization, albeit weakly and easily cancelled by an opposing intensity difference. A change of intercochlear phase difference will therefore be one of the factors determining the post-operative B.C. lateralization. It does not seem the only factor involved, however. Two findings at least cannot be explained by this factor alone, i.e., (*a*) the observation that replacement of the fixed stapes by a prosthesis in some cases leads to a simultaneous reversal of lateralization (simultaneous with the hearing gain), whereas in other cases a late or no reversal occurs, and (*b*) the observation that in some cases a reversal of lateralization takes place months after the definite hearing gain is attained.

3. *Loudness Habituation*

A third factor therefore seems to play a role. In our opinion, loudness habituation might be considered such a factor.

It is well known that after successful stapes surgery many patients experience sounds of moderate intensities with abnormally high loudness. It takes weeks or months before a normal loudness interpretation is attained. A period of habituation to the new range of sound intensities appears to be necessary. This phenomenon may be partially due to a middle ear and a cochlear dysfunction as a result of the recent surgery. A neurophysiological mechanism of habituation seems to be possible as well. In this connection we may point to the temporary loudness discomfort so often experienced by patients in whom a longstanding meatal obstruction is removed and by the hard-of-hearing in whom a hearing-aid is fitted for the first time.

A longstanding deafness leads to a relative sensory deprivation of the affected side. It seems not unlikely, therefore, that a sudden hearing improvement in these cases can cause a temporary imbalance of the central mechanism that evaluates the stimuli arriving from both cochleae. The information derived from

the ear that has been acoustically underestimated for many years, may be relatively overestimated during a certain period of time. If we accept the existence of such a mechanism the observation of a gradual reversal becomes more understandable.

ZUSAMMENFASSUNG

Untersucht wurde, wie bei Patienten mit einer doppelseitigen asymmetrischen Schalleitungsschwerhörigkeit als Folge von Otosklerose die Knochenleitungslateralisation (Weber) beeinflusst wird, wenn durch Stapeschirurgie das schlechtere Ohr in das bessere Ohr verändert wird. Bei 90 Patienten wurde die Knochenleitungslateralisation bei verschiedenen Frequenzen am Tage vor der Operation und an verschiedenen Zeitpunkten postoperativ — bis zu einem Maximum von vier Jahren gemessen.

Drei Arten postoperativen Verhaltens der Knochenleitungslateralisation wurden beobachtet:

1. „Gleichzeitiges Umschlagen" — die Lateralisation schlägt um auf das kontra-laterale Ohr, sobald das operierte Ohr besser wird als das nichtoperierte.
2. „Späteres Umschlagen" — die Lateralisation geht über in das kontra-laterale Ohr Wochen bis Monate nach dem Zeitpunkt, an dem das operierte Ohr die bessere Seite geworden ist.
3. „Kein Umschlagen" — die Lateralisation bleibt im operierten Ohr gerichtet, trotz der Tatsache, dass diese Seite nun das bessere Gehör hat. Es handelt sich um einen Weber-Paradox: Lateralisation nach der Seite mit dem geringsten Schalleitungsverlust.

Die Faktoren, die die Knochenleitungslateralisation bestimmen, wurden besprochen.

Der Einfluss von intercochleären Intensitätsunterschieden und intercochleären Phasenunterschieden wurde analysiert. Die Existenz eines dritten Faktors von „Zentraler Habituation" wurde vermutet. Da eine seit langem bestehende Taubheit zu einer relativen [illegible] des Sinnesorgans führen kann, ist es denkbar, dass eine plötzliche Gehörverbesserung eine zeitweilige Dysregulation des zentralen Ausgleichs der beiden cochleären Reize verursachen kann. Dies könnte die Erscheinung des langsamen und späten Umschlagens der Lateralisation erklären.

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HEARING IMPROVEMENT AFTER STAPES OPERATION IN RELATION TO VARIOUS DETAILS OF SURGERY

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A series of 354 stapedectomies performed by different techniques and selected from a larger material are presented. The hearing improvements obtained at different audiometric frequencies 6 months after surgery are discussed for the purpose of determining important details in postoperative sound conduction in operated middle ears. The following conclusions were drawn: (1) Good sound transmission was obtained by polyethylene prosthesis irrespective of the type of attachment to the incus. (2) No difference attributable to the type of material (polyethylene or stapedial crura) connecting the incus and the oval window was found. (3) Poorer results were reached by using the anterior than the posterior crus. (4) Complete fenestration of the oval window yielded better functional results than incomplete fenestration. (5) The results also appeared to be more favourable cases in which the fenestrated window was sealed some material compared with those in which was left unsealed.

After the first successful reports on stapes operations this kind of surgery has been used to restore the hearing of otosclerotic patients by various techniques. This surgery directed to the diseased area of the stapes itself has aimed to reconstruct a system capable of transmitting sound to the inner ear and including a functioning oval window and a connection from the incus to the window

The lines followed in the reconstructive surgery have been based on the knowledge of the normal middle ear mechanism. According to the present conceptions the middle ear acts as an impedance transformer matching the low impedance of the air to the high impedance of the inner ear fluids (Onchi, 1961 Møller, 1961 1963 1964 Zwislocki, 1962 Guinan & Peake 1966).

The normal sound conducting apparatus of the middle ear is a mechanical system composed of mass or inertia, stiffness, and friction or resistance. The inertia consists of the mass of the ossicles and of the tympanic membrane. The suspension ligaments of the ossicles including the annular ligament holding the stapes footplate in the oval window the attachments of the tympanic membrane and the tympanic muscles, all contribute to the stiffness of the system. Most of the inertia is due to the malleus and incus whereas the mass of the stapes amounts to only 1/20 of the whole inertia.

In such a system an increase in inertia increases the impedance at high frequencies. The impedance due to stiffness increases with decreasing frequency The resistive component of the system is independent of frequency

In the normal middle ear the ossicles do not vibrate as a single rigid body at least not in all frequency and intensity ranges. Especially the incudo-stapedial joint constitutes an elastic connection between the incus and the cochlea. In this joint there is loss of energy at high frequencies during sound conduction through the middle ear to the cochlea (Møller 1961).

In otosclerosis, bony fixation of the stapes footplate to the margins of the oval window limits the vibratory movements of the stapes.

This fixation increases the stiffness of the sound conducting mechanism and results in increased impedance and attenuation at lower frequencies. Thus, there is a loss at low frequencies in the earlier stages of the disease (Carhart, 1964). Higher frequencies are involved later. The conductive hearing loss amounts to some 55–60 dB when complete (Shambaugh & Carhart, 1951).

In otosclerotic surgery intervention in the region of the stapes results in changes in several of the details of the sound conduction apparatus. A new sound conduction system is created with differences especially in (1) the incudo-stapedial joint, (2) the mass, size, and elasticity of the stapes or its substitute if prosthetic materials are used, (3) the connection between the stapes and the cochlea, and (4) the size and function of the oval window. According to many of the clinical reports, this new sound conduction mechanism produces good functional results in 80–90% of the operated ears, although the artificial mechanism differs from the normal.

How does sound conduction take place in such an artificial system? Which of its details are of special importance for sound conduction after surgery? Answers to these questions have been sought in experiments on cadaver ears (Andersen *et al.*, 1964). In cadaver ears, however, the fibrous connections arising in the postoperative healing period are missing. Since, in clinical experience, great changes take place in hearing improvement during the first three postoperative months they must play a part in the new artificial sound conduction mechanism (Myers *et al.* 1959, Portmann, 1962, Rüedi & Suter 1962, Siirala *et al.*, 1969). A number of experiments have been conducted to clarify these various details by measuring cochlear microphonics or mechanical properties of the middle ear directly after application of different surgical techniques (Myers *et al.* 1959, Clark & Dunlop, 1968, Lawrence, 1960, Allen *et al.*, 1964, Cottle & Tonndorf 1966, Ronis, 1966). Measurements of the input impedance of the ear in operated patients can also provide us with information on the pathophysiological mechanism (Zwislocki, 1968).

One way of obtaining indirect information on the post-stapedectomy sound conduction is to analyse audiometric hearing improvements at different frequencies. A selective change in the vibratory mechanism of the middle ear can result in a specific high or low tone loss, which may remain undetected if only the mean of the speech frequencies is recorded in calculation of the hearing improvement.

The purpose of this paper is to present a series of patients operated on with different techniques and to draw conclusions by comparing the hearing improvements at different frequencies, as to the postoperative sound conduction.

MATERIAL AND METHODS

The study is based on 354 ears operated on in the period of 1961–1966 at the Helsinki University Otolaryngological Hospital by experienced surgeons. The material was selected from the total 1 014 otosclerosis operations. The cases fulfilling the following criteria were accepted. (1) operations performed by experienced surgeons, (2) control audiometric examination 6 months after surgery, (3) no previous operation in the ear concerned, and (4) a preoperative air-bone gap of at least 20 dB at the audiometric frequencies 500, 1,000 and 2,000 Hz or at least two of them.

Fig. 1 gives the age distribution of the patients. The various surgical techniques employed are presented in Table 1 in relation to the material used for connecting the incus to the oval window. Table 2 lists the type of oval window membranes used. The stapedius muscle had been sectioned in 191 of the 253 partial stapedectomies. A more detailed analysis of the surgical techniques is given elsewhere (Siirala *et al.* 1969).

The postoperative audiograms obtained 6 months after surgery were compared with the preoperative ones. The audiometric threshold values for different frequencies were recorded

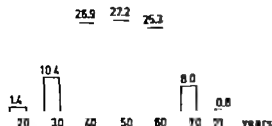


Fig 1 Age distribution of patients (%).

for computer analysis, done on an IBM 1620 digital computer For each audiometric frequency the following values were calculated (1) change in air conduction from pre to post operative, and (2) closure of air–bone gap as a difference between preoperative bone conduction and postoperative air conduction thresholds These two parameters, representing hearing improvement or loss, were tabulated in relation to the various details of surgery Significances were calculated in the case of large populations with a test of differences of proportions (Spiegel 1961) and in the case of small populations using hypergeometrical distributions (Documenta Geigy 1960)

RESULTS AND DISCUSSION

The *changes in air conduction thresholds* in the whole material 6 months after surgery are given in Fig. 2 The general trend is a decreasing gain and an increasing loss with increasing frequency This trend accords well with other reports in the literature (Tonndorf, 1959 Kos, 1962 Paparella, 1960 Colman, 1962 Schwetz, 1962 Stenger 1962 Shea & Sanabria, 1963 Ronis, 1966 among others). The high tone air conduction loss has been variously explained. Tonndorf (1959) attributes it to cochlear damage caused by high amplitude sudden transients during surgery Schwetz (1962) considers it associated with factors of the artificial ossicular chain, for instance the length of the prosthesis, rather than with cochlear damage. Shea & Sanabria (1962) believe it is due to a decreased mass of the artificial ossicular chain. On the contrary Stenger (1962) attributes it to an increase in mass and to a decrease of stiffness in the middle ear

Table 1 *Types of incus–oval window connections used*

	No. of ears	%
Anterior crus	17	4.8
Posterior crus	171	48.2
Anterior and posterior crura	65	18.3
Polyethylene prosthesis	101	28 7
Total	354	100 0

Table 2. *Grafting materials used to cover the oval window*

	No of ears	%
Fascia	319	90 1
Nothing	16	4.5
Other materials such as fat, vein, etc.	14	4.0
Not stated	5	1 4
Total	354	100.0

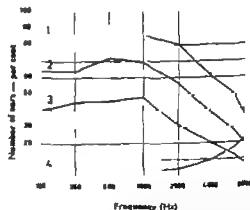


Fig 2 Graph showing air conduction changes in the whole material (%) at different frequencies. The curves indicate gains of more than 10 dB (curve 1), more than 20 dB (curve 2), and more than 30 dB (curve 3), and losses of more than 10 dB (curve 4) Thus, for instance, some 85% of the whole material reached an air conduction gain of at least 10 dB up to 1 000 Hz, as shown by curve 1

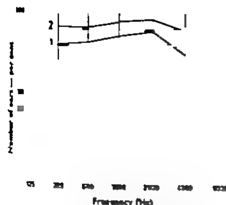


Fig. 3 Graph showing closure of air-bone gap [...] hole material (%) at different frequencies. (The closure of the gap is taken as the difference between postoperative air conduction and preoperative bone conduction values at each frequency.) The curves show the percentage of overclosure and closure to 10 dB (curve 1) and ≤ 20 dB (curve 2).

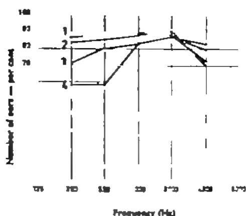


Fig. 5 Graph showing overclosure and closure [...] air-bone gap to 10 dB or less (%) at different frequencies using polyethylene (curve 1), posterior crus (curve 2), anterior and posterior crura (curve 3) and anterior crus (curve 4).

The number of patients reaching a gain in air conduction threshold declines (Fig. 2) after 1,000 Hz, whereas, if the closures of the air-bone gap are studied (Fig. 3) good closure percentages are obtained even at 2,000 Hz. Thus it may be that the small proportion of gains in air conduction at high frequencies does not indicate any true loss. The preoperative air-bone gap is greater at lower frequencies, since otosclerosis first produces a low frequency conductive hearing loss. Therefore

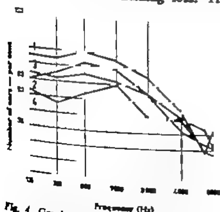


Fig. 4 Graph showing air conduction gains [of] 20 dB or more (%) at the different frequencies using the following methods: polyethylene prosthesis (curve 1), posterior crus (curve 2), anterior and posterior crura (curve 3), and anterior crus (curve 4).

air conduction cannot be expected to improve so much.

Three *types of attachment* were used with polyethylene prosthesis: (1) no special measures for attachment, (2) the so-called "wing-grip" type (Siirala *et al* 1962), and (3) the so-called self-locking -type (Siirala, 1965) Comparison of these three types of attachment showed no significant differences as regards gain in air conduction threshold. There are several reports concerning the coupling between the prosthesis and the incus (e.g. Myers *et al* 1959 Goodhill, 1962) In the experimental study of Andersen *et al* (1964) jumping and dislocation of the prosthesis could be demonstrated especially at high sound pressures. Audiograms, however are taken at low sound pressure levels. In addition, the experiments of Andersen *et al* were made on cadaver ears, in which the fibrous connective tissue tent growing around the union of the prosthesis and incus postoperatively was, of course, absent.

When the threshold changes were compared in respect of the *various connections used between the incus and the oval window* the results shown in Figs. 4 and 5 were obtained for gains in air conduction and closure of air-

bone gap respectively It was found that the anterior crus is inferior to the other techniques at lower frequencies (250 and 500 Hz) a difference reaching 0 05 level of significance

The difference between the techniques using posterior and anterior crura is mainly in the type of connection formed at the incudostapedial joint between the stapes and the incus. A loose linkage at the joint may result in decreased sound conduction as demonstrated by Clark & Dunlop (1968)

The elastic modulus reported for polyethylene is about $4.5 \times 10^{4} N/m^{2}$ and thus remains about 10^{6} times smaller than that reported for bone tissue (according to Carothers *et al* 1949 $2.7 \times 10^{10} N/m^{2}$) However according to the present results, no such differences are obtained as might be accounted for by the difference in stiffness It may well be that the normal stapes is even stiffer than necessary for good sound conduction.

The prosthetic materials used do not differ significantly from the normal stapes as regards mass (polyethylene tubing, about 5 mm in length, has a mass of 4 mg, whereas, according to Kirikae, 1960 the mass of the normal stapes is about 3 mg) Thus the difference between the masses is so small that no differences in hearing improvement can be expected on that basis. According to a number of experimental studies, small differences in mass do not matter while a greater increase results in attenuation at high frequencies (Lawrence, 1960 Andersen *et al* 1964 Kirikae *et al* 1964 Cottle & Tonndorf, 1966)

On the whole, as regards the incus–oval window connection, the present results agree with other clinical reports there was no obvious tendency for any one material to provide better gains (e.g. Myers, 1962 Kaplan & Shambaugh, 1961 Plester 1963 Salvá, 1964)

The comparison of cases with *a sectioned and an intact stapedius muscle* showed no statistically significant differences in hearing improvement. The present results, however are based on hearing tests at rather low sound pressure levels, where muscle contraction does

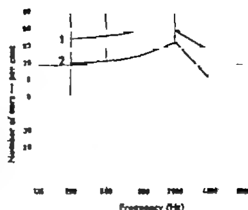


Fig 6 Graph showing overclosure and closure of air–bone gap to 10 dB or less (%) at different frequencies obtained by complete fenestration of the oval window (*circle 1 212 ears*) and by incomplete fenestration (curve 2, [illegible] ears).

not yet take place. This may explain the loss of difference.

In the region of the *new oval window* several details of importance should be considered, such as: (1) the size of the fenestrated window (2) the type of grafting material, (3) the type of coupling between the oval window membrane and the prosthesis, and (4) the size *of the tip of the prosthesis or stapedial crus* in relation to the size of the membrane (v Békésy 1960)

Complete opening of the oval window gives somewhat better closure of the air–bone gap at all frequencies (to 0 05 level of significance) than does incomplete opening (*Fig. 6*) Sufficiently extensive fenestration is required to provide a great enough volume displacement of the inner ear fluids. The results are thus in agreement with those of Heermann (1960) and Hlaváček (1967).

Operation without grafting material seems to offer a smaller chance of good gain at low frequencies (Fig. 7) than the use of fascia, for instance A thin membrane is formed on the oval window even when no grafting is used (Goldman *et al* 1963 Thomas & Liu, 1967). However the vibratory properties of such a membrane may not be as good as after application of some grafting material. Unfortunately

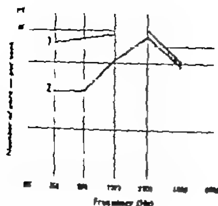


Fig. 7. Graph showing overclosures and closures of the air-bone gap to 10 dB or less (%) at the different frequencies obtained with fascia grafting (curve 1) and no grafting (curve 2).

grafting materials other than fascia were used too infrequently to make comparisons possible.

CONCLUSIONS

The data reported on the normal function of the middle ear mechanism have made it possible to analyse circumstances in which the normal function is altered either by a disease like otosclerosis or by surgery. It has long been known that, to obtain a successful result in stapes surgery, it is necessary to restore the function of the oval window and to re-establish a connection between the incus and the fenestrated window.

The choice of the surgical technique depends, of course, on factors other than those affecting the sound conduction properties of the various materials. A good functional result can be obtained if the fluid volume displacement at the oval window is large enough. However the function of the new oval window sealed by a grafting membrane is a complicated issue and difficult to control in surgery. In addition, the postoperative healing processes contribute their own share.

In summary the present study, designed to determine the effect of various surgical details on the hearing improvement, allows the following conclusions to be drawn.

1. The different types of attachment used in this study between the polyethylene prosthesis and the incus all ensure good sound transmission.

2. The different stiffness of polyethylene prosthesis as compared with the stapes does not cause any difference in sound conduction in the middle ear after surgery.

3. Poorer low frequency results are obtained with the anterior crus than with other techniques.

4. Small changes in the mass of the incus-oval window connection seem to play no role.

5. The functional state of the stapedius muscle has no effect on sound transmission after surgery at threshold sound pressure levels.

6. From the point of view of sound transmission, complete fenestration of the oval window appears to be better than incomplete fenestration.

7. A better hearing improvement is obtained by sealing the fenestrated oval window with some material than by leaving it unsealed.

ZUSAMMENFASSUNG

Eine Serie von 354 Stapedektomien, die ein Teil eines nach verschiedenem Verfahren operierten Materials ist, wurde analysiert. Die Hörverbesserung bei verschiedenen Frequenzen wurde 6 Monate postoperativ berechnet um wichtige Einzelheiten der Schallübertragung nach Mittelohreingriffen festzustellen. Es wurde gefolgert: 1. Eine gute Schallübertragung wurde mit einer Polyäthylenprothese unabhängig vom Anheftungstyp am Amboss erzielt. 2. Kein Unterschied, der auf der Wahl des Prothesenmaterials beruhte (Polyäthylen oder Stapesschenkel), konnte ermittelt werden. 3. Die Operationsergebnisse waren besser mit hinteren als mit vorderen Schenkel. 4. Bei vollständiger Fensterung des ovalen Fensters lagen bessere Resultate vor. 5. Die Ergebnisse waren noch vorteilhafter falls das Fenster mit irgendeinem Material gedeckt wurde.

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DEVELOPMENT OF DICHOTIC AND MONAURAL HEARING ABILITIES IN YOUNG CHILDREN

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Paired spoken words were presented to 80 normal children ith range from 3 to 6 years and 5 young adults in such a way that different words arrived simultaneously at the two ears (dichotic listening) and at the one ear (monaural listening) to observe the development of dichotic and monaural separation abilities. The results demonstrated that in case of dichotic listening, significantly more words were recognized from the right ear than from the left ear. On the other hand, in monaural listening the results were almost identical for each ear. The monaural separation ability is inferior to the dichotic separation ability in all age groups. Dichotic and monaural separation abilities develop as with ageing. Six-year-old children and young adults achieve almost identical results. In group age 3 girls are superior to boys on both dichotic and monaural separation abilities but there is no difference between boys and girls in the older age groups.

Several lines of evidence suggest that speech perception is characterized by a process different from that for the perception of other sounds. Ohta *et al.* (1967) classified the phenomenon of binaural hearing: binaural summation, binaural separation and binaural fusion. Binaural separation means the discrimination in each ear at "dichotic" listening. Many investigations have been carried out on binaural separation ability using dichotically presented speech sounds to study cerebral dominance (Kimura, 1961 *a*, *b* Dirks, 1964 Inglis, 1965). These studies with adults had shown that digits arriving at the right ear are more accurately reported than those at the left. This effect depends on the fact that speech is represented in the left hemisphere. Kimura (1963) tested dichotic listening of children from age four upwards and found both boys and girls showed a significant right-ear superiority.

The author has investigated the development of central auditory function concerning speech development in young children. The present experiment was designed to elucidate the development of dichotic and monaural separation abilities in young children using dichotic and monaural listening. The term "dichotic listening" means the simultaneous presentation of two different words to the two ears and "monaural" listening means the simultaneous presentation of two different words to one ear only (Fig. 1).

METHODS

Subjects

The subjects were 80 normal children, aged 3–6 years. An attempt was made to have 10 subjects in each age-sex grouping to keep the number of boys and girls equivalent. Every attempt was made also to keep the sample unselected, with the reservation that children with hearing disorders or mental retardation were not tested. In preliminary tests, each subject was screened audiometrically at a hearing level of 20 dB at the frequencies of 1000 Hz and 4000 Hz. All subjects had above-average IQ's. To obtain a comparison with children, 5 young adults with normal hearing were selected 2 females and 3 males with mean age of years

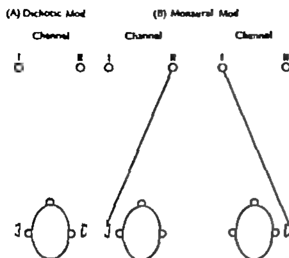


Fig 1 Modes of presentation of the paired words to the ears.

Test material

The test material consisted of 20 two-syllable words and 20 three-syllable words, which were considered to be relatively homogeneous in intelligibility for young children. Those words were recorded to pair each syllable word separately on a dual-channel Sony TC 9680 tape recorder so that both words began at exactly the same time The first channel consisted of 0 two-syllable words and 10 three-syllable ds, and the remainder on the second channel. The pairs of words from a dual-channel appeared at 5 sec intervals. Ten paired words of each syllable were divided into two groups, *that is, each group consisted of 5 paired words.* Five pairs of words were presented at each set volume, making a total score of 5 for each ear Taped words were delivered to the subjects through a dual-channel tape recorder via a speech audiometer with stereophonic ear phones. Stimuli in both ears were similar

Procedure

Each subject was tested in a sound-proof room. The threshold for spondee words with speech audiometer (67 AB list of Japanese Audiology) was obtained on each ear of a subject by up-and-down method. In this investigation, the spondee words were employed only as reference to establish the level at which the experimental lists were to be delivered. Subsequently all test words were delivered to the subject's ears at sensation level above the obtained spondee thresholds, with range from 20 dB to 80 dB with 10 dB steps. Each subject received a series of 5 paired words at each set volume. The subject was instructed to report what he heard. For half the subjects in each age-sex group the earphones were reversed so that any remaining asymmetries in the tape or apparatus were counterbalanced

First, the dichotic listening test was carried out and the levels were determined which were sufficient for each subject to score 5 (100%) on the words tests. Secondly at the minimal level at which the score of dichotic listening achieved 5 for both ears, the monaural listening test was done for each ear

RESULTS

First, the dichotic separation ability was investigated. The number of correct responses on each test was checked. The differences of

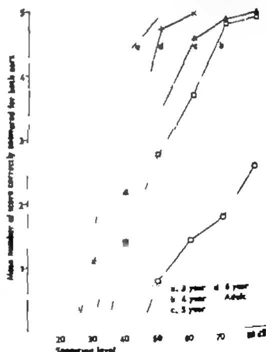


Fig 2 Dichotic separation ability of two-syllable paired words.

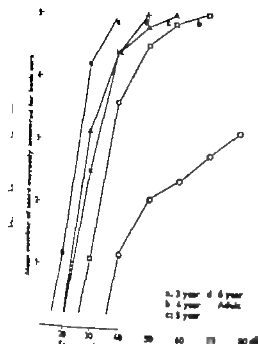


Fig. 3 Dichotic separation ability of three-syllable paired words.

the mean scores in each age group are shown in Figs. 2 and 3 It is apparent from these figures that the higher the age, the better the dichotic separation ability The hearing level for three-syllable paired words in listening was about 10 dB lower than for two-syllable paired words. The 3-year-old subjects had great difficulty in obtaining total score 5 even at 80 dB, whilst 50 dB was sufficient for young normal adults to achieve total score 5

When the total score achieved on the test is considered, boys were inferior though not significantly to girls at age 3 but the difference between boys and girls decreased in the older age groups. The difference between scores for the two ears were compared by use of t test. The results are shown in Tables 1 and 2 They showed that in general, especially in boys there is a significant difference in favor of the right ear Normal young adults obtained near perfect scores at a lower sensation level than in the case of young children, so that the dominance effect was not always observed as clearly as in young children.

Table 1 *Mean number of scores for each age sex group in dichotic listening (boys)*

Age (years)	Syllable	Right ear	Left ear	Bal (R 1		P
3	2	3.2	1.8	1 4	2 7	01
	3	2.7	1 6	1 1	87	01
4	2	1.5	1 1	0 4	0 73	
	3	1 4	0.8	0 6	15	0
5	2	1 6	0 4	1.[illegible]	45	01
	3	1.5	1.0	0.5	0 7	
6	2	2.2	1.0	1.[illegible]		
	3	1 7	0 4	1.3		

Secondly the monaural separation was investigated The mean scores ach correctly at each ear in each age–sex gr are shown in Figs. 4 and 5 Three-syll paired words were more correctly discriminated than two-syllable ones. The monaural separation ability increased with age. Moreover there is almost no difference between boys and girls in this respect. The monaural separation scores from the right ear generally maintained a slight superiority but the difference between sides did not reach a level of statistical significance

In a comparison of dichotic listening and monaural listening, it was found that when the paired words were presented dichotically words arriving at the right ear were more efficiently (statistically significant) recognized than words arriving at the left ear and when the same paired words were presented monau-

Table 2. *Mean number of scores for each age–sex group in dichotic listening (girls)*

Age (years)	Syllable	Right ear	Left ear	Balance (R L)	t	P
3	2	3.2	2.3	0 9	1.20	
	3	2.7	1.0	1 7	7 77	< .001
4	2	1 1	1.3	−0.2	0.52	
	3	1 1	1 1	[illegible]		
5	2	2.0	1.0	1.0	2.17	< .05
	3	[illegible]	0.3	1.5	2.35	.05
6	2	1 9	1 1	0 8	1.49	
	3	2.0	1.4	0 7	0 87	

Each age-sex group consists of 10 subjects.

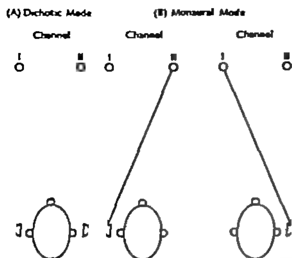


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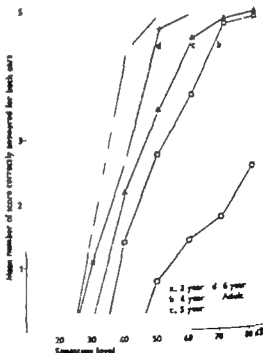


Fig 2 Dichotic separation ability of two-syllable paired words.

even when it occurs as late as age 6, does not permanently affect speech. Zangwill (1960) proposed that left cerebral dominance is established gradually during childhood. In dichotic listening, boys are significantly better on the right ear than on the left at the age of 3. Although no significant sex difference was obtained for the right ear effect in the age range above three, there is a little difference between boys and girls in overall efficiency on the task. The present investigation suggests that boys at age three lag behind girls in the perception of speech sounds. Terman & Tyler (1954) stated that concerning sex difference in cognitive ability girls excel boys in about all speaking skills at least in the early years. McCarthy (1930) suggested that with regards to language ability girls go through the developmental cycle more rapidly than boys, but both arrive eventually at approximately the same level.

The simultaneous dichotic presentation of words produced a right ear effect. In normal subjects above 3 years old, the discrimination of dichotic listening obtained by each 5 paired words was close to 100% at a satisfactory level. When the same paired words were delivered monaurally, the discrimination scores were almost identical for each ear and attained to only 50% except young adults and 3-year old children. One obvious implication based on the present study is that responses from tests with monaural paired verbal materials are not demonstrably affected by dominance for speech. Lindén (1964) reported that the intelligibility for monaural distorted speech is the same in both ears and suggested that the reduction of intelligibility for distorted speech is due to the reduction of the ability of the auditory center. The author deduced that monaural listening of paired words is considered to be the same as the intelligibility test by distorted speech and there is a difference in mechanism between dichotic separation ability and monaural separation ability.

Ohta (1966) suggested that the ability of binaural separation is a function of the highest levels of the central auditory pathway. Antonelli & Calearo (1968) deduced that a lesion of the brain-stem reticular formation causes a decrease of the ability for temporal discrimination of auditory messages.

The results of the present investigation contribute to an understanding of the development and impairment of central speech and auditory system.

ZUSAMMENFASSUNG

Der Hauptzweck dieser Untersuchung war die Entwicklung des dichotischen und des monauralen Diskriminationsvermögens durch dichotische und monaurale Darbietung gepaarter Wörter an beiden Ohren bei jungen normalen Kindern im Alter von 3 bis 6 Jahren, zu zeigen.

Alle Personen konnten die Prüfwörter bei dichotischer Darbietung besser verstehen als bei monauraler Darbietung. Die Ergebnisse bei dichotischer Darbietung zeigte, dass signifikant mehr Wörter vom rechten Ohr aufgefasst wurden als vom linken Ohr. Doch bei monauraler Darbietung waren die Ergebnisse für jedes Ohr identisch. Bei 3-jährigen Kindern konnten Mädchen die Prüfwörter besser verstehen als Knaben. Dichotisches und monaurales Diskriminationsvermögen zeigten die chronologische Entwicklung.

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THE ULTRASTRUCTURE OF THE HUMAN STRIA VASCULARIS PART I

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The human stria vascularis contains three types of cells (marginal, intermediate and basal) which are remarkably similar to those found in the smaller animals. The marginal cells are rich in a homogeneous electron-dense substance, and contain numerous coated vesicles, mitochondria, rough endoplasmic reticula, and free RNA particles. The luminal surface often shows microvilli and a rather thick unit membrane. Numerous cell processes extend deeply toward the base, interdigitati g with those of the intermediate and basal cells. The basement membrane is generally absent except in areas adjacent to the blood vessels. The intermediate cells are rich in Golgi network, smooth and rough endoplasmic reticula, and large mitochondria. Peripheral to their cell bodies are often short segments of the basement membrane. The basal cells are flat and long and show numerous, short desmosomes and fasciae and maculae occludentes. They send cell processes into the other strial epithelia but desmosomes between them are few The junction between the basal cells and fibrocytes of the spiral ligament shows desmosomes at random points, and the intercellular space of fibrocytes contains numerous bundles of fibrils which are rectangular in cross section. The blood vessels of the stria vascularis lack smooth muscle cells and neural elements, and about both mar ginal and intermediate cells.

The purpose of the present study is to describe the morphology of the stria vascularis from human ears presenting the typical functional alterations of Menière s disease. This first paper will be a description of the strial cells which we consider normal or nearly normal. Other papers will follow with ultrastructural descriptions of the pathological changes.

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MATERIALS AND METHODS

The tissues for this study were obtained during surgical procedures being performed to ablate vestibular function. Each patient had disabling episodic vertigo as well as unilateral severe hearing loss for more than one year Auditory and vestibular tests showed the opposite ears to be normal or near normal. The surgical procedures on the affected ears were performed with the patients under general anesthesia and consisted of the following steps:

1 Infiltration of the skin of the external auditory canal with 1% xylocaine containing adrenalin (1 1000)
2. Elevation of a tympanomeatal flap
3 Removal of the incus and stapes
4 Removal of part of the lateral wall of the otic capsule and placement of the specimen in fixative solution
5 Removal of segments of the membranous labyrinth and placement in fixative solution

The excised fragment consisted of the bone between the oval and round windows and adjacent promontory attached to which were the stria vascularis and spiral ligament of the basal 5 to 7 mm of the cochlea. In ten of thirteen specimens the stria vascularis remained at tached to the spiral ligament and was available for study All specimens were fixed in 1% phosphate buffered osmium, dehydrated in alcohol, imbedded in Epon, cut with an LKB ultrotome double stained with uranyl and lead citrate, and photographed w mens Elmiskop 1



Fig 1 A low magnification of the stria vascularis showing marginal cells (*M*), capillary (*C*), intermediate cells (*IN*), and basal cells (*BA*). Note extensive interdigitation of cell processes at the lower left corner 1,050.

FINDINGS

The stria vascularis of the human contains three types of cells (Fig. 1) similar to those described in small animals, marginal, intermediate and basal cells. Although these specimens came from inner ears manifesting abnormal function, a relative distinction between the normal and pathological ultrastructures can be made by comparison with the stria vascularis taken from early post-mortem human specimens, Rhesus and squirrel monkeys, guinea pigs, bats, opossums and cats.

Marginal cells (chromophil cells dark cells)
The marginal cells are noted by their dark appearance which is due to numerous cell organelles and an electron-dense, diffuse substance spread throughout the cytoplasm. Their nuclei are located rather high near the cell apex and are marked by numerous invaginations. The central and basal cytoplasms (about /3 of the cell height) show extensive plasma membrane infoldings which interdigitate with those of the intermediate and basal cells.

The luminal surface of the marginal cell shows a varying number of short microvilli

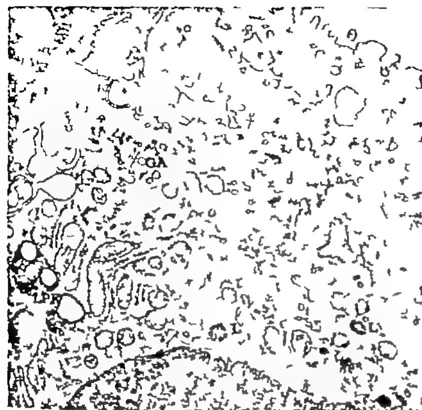


Fig. 2. A high magnification of the apex of the marginal cell, showing short microvilli, pinocytotic vesicles, Golgi apparatus (*GA*), free RNA particles, rough endoplasmic reticulum, lysosomes (*L*), and dense bodies, presumably lipofuscin granules (*LPF*). Mitochondria are located some distance away from the luminal surface. 26,660

(Figs. 1, 2, 3 *C*) and pinocytotic invaginations which are coated with a diffuse substance. The number of these vesicles in the cell varies. In some cells the pinocytotic vesicles are great in number while in adjacent cells they are few. The unit membrane at the endolymph surface often appears thicker than that of the deeper part of the cell. The inner leaflet of the unit membrane appears to be denser and perhaps thicker in comparison to the outer leaflet at both luminal and interdigitating cell surfaces (Figs. 3 *A*, *B*, *C*).

Directly beneath the luminal plasma membrane there is often a thin layer of electron-dense substance running parallel to the surface and extending into the microvilli where cores are sometimes demonstrable. Close to the luminal surfaces are also extensive networks of tubules which contain a diffuse substance. One, two or three centrioles are rather commonly observed in this area, sometimes they are associated with rootlets running diagonal to the free cell surface. The Golgi apparatus is small, and there are several. At their adjacent areas are small and large dense oval bodies with a distinct limiting membrane which is underlined by a circular layer of light band. The substance within these small bodies is homogeneous, however some of them contain short membranous segments. They are presumed to be lysosomes (Fig. 2). The large bodies containing a similar substance and lipoidal droplets and showing pleomorphism are probably lipofuscin granules (Fig. 2). Free RNA particles, rough endoplasmic reticula and mitochondria are numerous in the apical cytoplasm. A few multivesiculated bodies are also found near the endolymph surface.

The lateral plasma membranes establish numerous interdigitations with adjacent marginal cells and their cell border lines are ... cult to establish. Large cell processes ... mitochondria, free RNA and ... The small cell processes show a

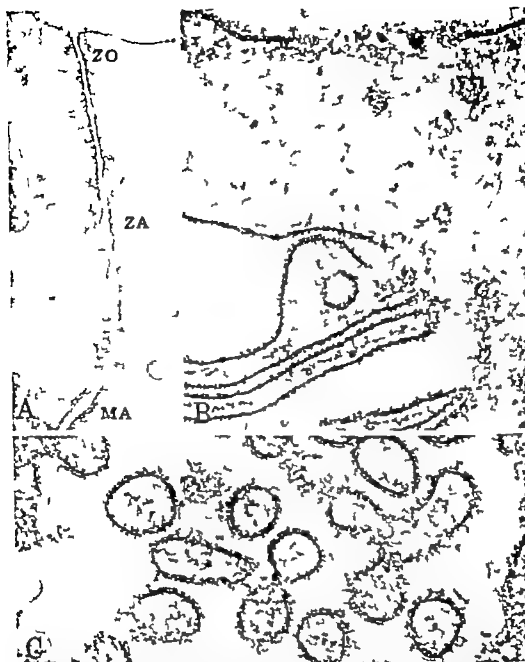


Fig 3 High magnification of the luminal surface of marginal cells. (*A*) Cell junction between marginal cells. At the zonula occludens (*ZO*) pproximation of the outer leaflets is faintly shown. Zonula adherens (*ZA*) and macula adherens (*MA*) 68,000. (*B*) The inner leaflet of the unit membrane ppears more opaque than the outer leaflet at both the endolymph surface and interdigitating cell processes. The unit membrane at the luminal surface appears ider in some areas than in the cell processes located in the deeper part. Not the thin homogeneous layer in the cell processes. 122,000 (*C*) A cross section of mic rovilli of the marginal cells. Note the denser inner leaflet of the unit membrane. 124,000.

homogeneous substance running parallel to the surface membranes (Fig. 3 *B*) Desmosomes are rare between the lateral pr of the marginal cells but are common n umi- nal surface where zonula occluden n cula adherens are observed (Fig. 3 *A*) Desmosomes are extremely rare between the marginal and intermediate cells, and are also scarce between the marginal and the basal cells (Fig. 5 *A*)

The basement membrane between the mar



Fig. 4 Electron micrographs of intermediate cells (*IN*). (*A*) The cytoplasm contains a few Golgi apparati, numerous tubules and mitochondria which are larger than those seen in the marginal cell processes (*M*). Note the basal cell extensions (*BA*) among the marginal and intermediate cells. 10,000 (*B*) The junctions between the marginal cells (*M*) and intermediate cells (*IN*) often show thin homogeneous layer similar to the basement membrane and cytoplasmic condensation adjacent to this layer (*arrows*). Such layer is often located closer to the intermediate cell than the marginal cell. 12,000

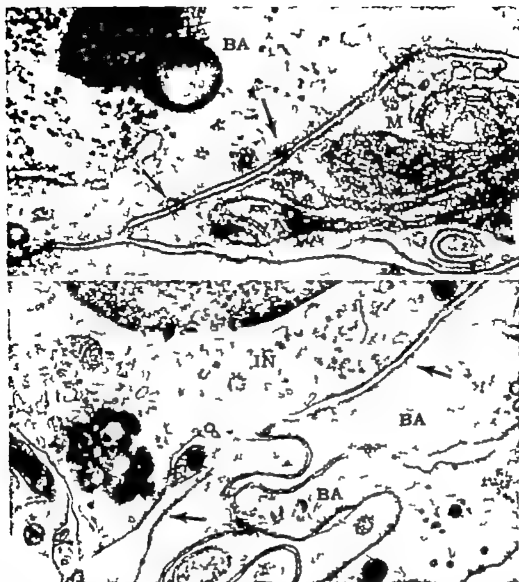


Fig 5 Strial cell attachments to the basal cells. (*A*) Two localized cytoplasmic condensations (*arrows*) between the basal cells (*BA*) and marginal cell (*M*). The intercellular distance is 115 to 150 A. The large, dense body in the basal cell is presumably a lipofuscin granule. 52,000. (*B*) Cytoplasmic condensations in the basal cell (*BA*) and intermediate cell (*IN*) adjacent to the homogeneous thin layer (*arrows*). The intercellular gap at the right is about 450 A. 24,000.

ginal cells and the basal cells is rare. However short basement membranes are frequently observed between the marginal cell and intermediate cell in which cytoplasmic condensation is frequently seen on both sides, usually more condensation on the side of the intermediate cell. The basement membrane is sometimes found below the marginal cell adjacent to the capillary wall

Intermediate cells (chromophobe cells light cells)

The intermediate cells are common but less in number in comparison to the marginal cells. They are located below the marginal cells, and their cell processes interdigitate with those of the marginal cells and basal cells. Their cytoplasm appears lighter than that of the marginal cell due mostly to the less dense ground

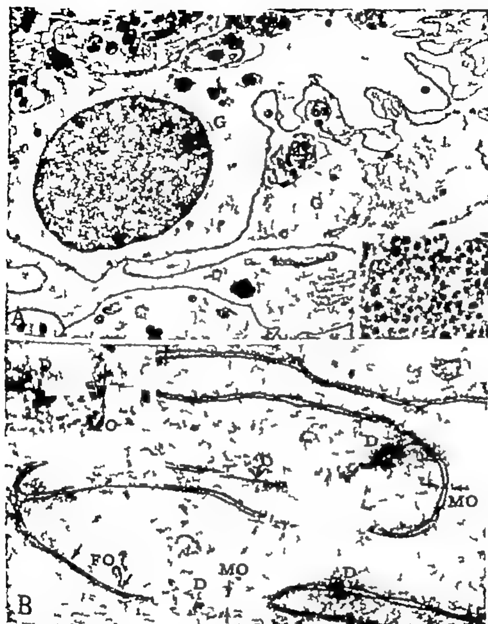


Fig. 6 Basal cells of the stria vascularis. (*A*) The basal cells contain numerous filaments, rough endoplasmic reticula and, occasionally masses of glycogen granules (*G* see inset). The intercellular junction between basal cells is narrower than that seen between marginal and basal cells. 8,400. (*B*) A high magnification of basal cells showing cell junction and desmosomes. 71 000. Fascia occludens (*FO*) is extensive with an 80 Å distance between the inner leaflets, macula occludens (*MO*) is short with about 70 Å gap Desmosome (*D*).

substance Mitochondria are larger in diameter and their matrices are lighter than those of the marginal cells (Fig. 4 *A*) Free RNA particles and rough and smooth endoplasmic reticula are scattered throughout the cytoplasm. Golgi apparatus is prominent, a large, whorly smooth endoplasmic reticulum is sometimes present. A few multivesiculated bodies as well as some small dense bodies and large containing lipoidal droplets are



Fig 7 Transitional area between basal cells (*BA*) and the fibrocytes (*F*) of the spiral ligament. A clear differentiation between the basal cell and fibrocytes is often difficult. Note the wide space in the spiral ligament in which bundles of fibrils are scattered, while the space between the basal cell layers is narrow Cytoplasmic condensations (*arrows*) are observed adjacent to the bundles of fibrils and basement membrane-like layer 18,060.

centriole is common and, in some instances, is in the form of a rudimentary cilia projecting into the intercellular space The cell junction between intermediate cells shows a few desmosomes (Fig. 8 *B*) and small segments of the basement membrane. Between the intermediate and marginal cells, the short basement membranes are often noted intermittently around the perimeter of the intermediate cell (Fig. 4 *B*) Cytoplasmic condensation is usually present adjacent to the basement membrane which is located equidistant or closer to the intermediate cell than the marginal cell. Similar junctions are also found between the intermediate and the basal cells (Fig. 5 *B*)

Basal cells

The basal cells are flat and long. There are one to several layers which run parallel to the luminal surface below the marginal and intermediate cells (Figs. 1 6) In general the cytoplasm is light with cell organelles, though the cells adjoining other strial cells often show more mitochondria, rough endoplasmic reticula, free RNA and tubules near the oval or flat nucleus. The major part of the cytoplasm is frequently filled with short filaments (Figs. 6 *A* *B*), which make the cells appear dense. These cells often contain huge, irregular masses of coarse granules mixed with lipoidal droplets, and sometimes a large concentration



Fig 8 (*A*) A capillary (*C*) is surrounded by concentrically arranged basal cells (*BA*) which depart from the usual pattern of parallel orientation at the luminal surface (*arrow*). Note bundles of collagen fibrils (*CO*) at the perivascular area. A high magnification of such fibrils is shown in Fig. 9 *B* 5,300.

(*B*) Both marginal cells (*M*) and intermediate cells (*IN*) re directly exposed to the capillary (*C*) The endothelial cell are surrounded by layers of base ment membrane and homogeneous substance Desmosomes between the intermediate cells are shown on the left side (*arrow*). Pericyte (*P*). 1_,700.

of glycogen granules (Fig. 6 *A*) The basal cells send cell processes usually a short distance toward the marginal cells in an irregular manner The basal cells adjacent to the spiral ligament often look like the fibrocyte. The fibrocytes of the spiral ligament near the basal cells are loosely organized in comparison to the com pactly arranged strial epithelium (Fig. 7) Among the basal cells and fibrocytes are numerous fibrils imbedded in a homog

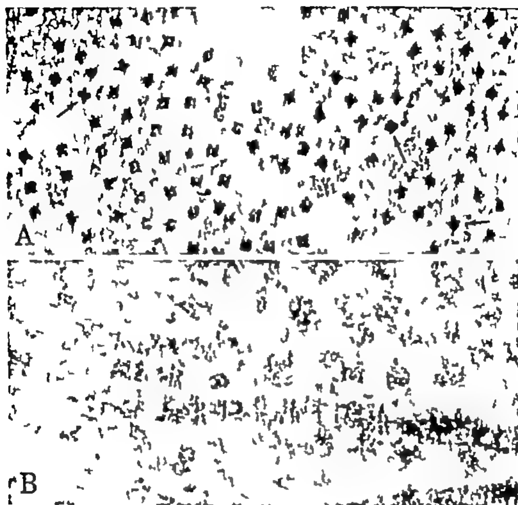


Fig 9 Both photographs are taken with the same [magni]fication. ×60,000 (*A*) Cross section of fibrils in a matrix of homogeneous substance among fibrocytes of the spiral ligament. Each fibril is rectangular in shape and consists of subfibrils whose number varies, but four units, one at each corner are the most obvious (*arrows*). The fibril measured 108 A on the side and is surrounded by light zone. (*B*) Large fibers, collagen type, are found adjacent to the strial blood vessels. Note periodicity of bands across the fiber Diameter is about 250 A.

substance The fibrils are rectangular in cross section, measure 108 A on the side, and consist of a varying number of subfibrils, but one at each corner of the square is most obvious (Fig 9 *A*) as demonstrated in the spiral ligament of the Rhesus monkey by Takahashi & Kimura (1970) and in the vestibular perilymphatic tissue of the rat by Hamilton (1967)

One of the most striking features of the basal cell layer is an extremely narrow intercellular space and the presence of numerous short desmosomes (Fig. 6 *B*) There are two types of close junctions, the fascia occludens with an 80 A gap and the shorter and narrower macula occludens with a 70 A gap between the inner leaflets. The cell junction between the basal cell and the fibrocyte is similar but the number of attachments is far less in comparison to the basal cell group Although the fibrocytes of the spiral ligament are widely spaced, their long cell processes extend in all directions and establish many desmosomes and fasciae occludentes at their cell junctions. Occasionally a thin, short basement membrane-like substance is seen adjacent to the fibrocyte The cytoplasm is condensed adjacent to this layer

as well as in the area where the bundle of fibrils approximates the cell wall (Fig. 7). The cell junction between the basal cell and other strial cells is described in the previous section.

Blood vessels

Blood vessels penetrate the basal cell layers and run among the marginal and intermediate cells. Smooth muscle cells and neural elements have not been identified in the strial epithelium. However they may be partially or completely surrounded by concentrically arranged basal cells (Fig. 8 *A*). The endothelial cells are not fenestrated, though in some areas they are extremely thin. Pinocytotic vesicles are demonstrated more often on the basal side. The nucleus is indented and flat, in the perinuclear zone are a few mitochondria, some tubules, RNA particles and often a centriole. The cytoplasm contains a few lipoid droplets, dense bodies and some localized filaments. Cytoplasmic condensation is noted at the endothelial cell junction where approximation of the outer leaflets of the unit membrane takes place.

The basal part of the endothelial cell is underlined by a basement membrane which is approximated by the cell processes of pericytes which are likewise surrounded by the basement membrane. Discontinuity of the basement membrane is observed only at the junction between the endothelial cell and the cell processes of pericytes at which point a pericyte cytoplasmic condensation is common. Peripheral to the endothelial cells are also a series of basement membrane-like layers, a thick layer of homogeneous substance in which are filaments, large dense bodies without the limiting membrane, and groups of large fibers which show occasional periodicity and are presumed to be collagen (Fig. 9 *B*). Both marginal cells and intermediate cells are directly exposed to the capillary walls (Fig. 8 *B*).

DISCUSSION

The ultrastructure of the cells of the stria vascularis has been studied for many years in guinea pigs (Engström *et al.* 1955, Smith, 1957, Rodriguez-Echandia & Burgos, 1965), cats (Hinojosa & Rodriguez Echandia, 1966) and mice (Spoendlin, 1967). The cytoarchitectures differ somewhat in different species, but consistent agreement is noted in the presence of three types of cells, marginal cells near the endolymph surface, intermediate cells below the marginal cells, and basal cells adjacent to the spiral ligament. In comparison to adult animals, the marginal cells of man frequently show short microvilli at the luminal surface. The presence of such microvilli in other post mortem stria suggests that they may be a rather common occurrence in the human. Associated with the microvilli are numerous pinocytotic vesicles, which reinforces the concept of fluid resorption, at least in part, at this site (Hinojosa & Rodriguez Echandia, 1966). A completely opposite interpretation of these vesicles is made by Johnson & Spoendlin (1966) who believe that the vesicles carry potassium into the endolymph. The same authors further state that secretory granules are discharged into the endolymph. In our material similar granules are observed, but are interpreted to be lysosomal granules with no evidence of such granules being directly discharged into the endolymph. At present, the role of marginal cells is not clearly established, but they are presumed to take an active part in both fluid and electrolyte transport.

Equal thickness of the outer and inner leaflets of the luminal membrane and the thicker and denser inner leaflet of the lateral and basal surfaces of absorptive cells are noted by Farquhar & Palade (1963). Johnson & Spoendlin (1966) report similar findings in the marginal cells of guinea pigs. In our human specimens, the outer leaflets at both the luminal and intercellular surfaces appear less dense than the inner leaflets. Although the inner leaflet stains denser, the actual thickness may be equal or slightly greater than that of the outer leaflet of both of these cell surfaces. The entire width of the unit membrane at the luminal surface is often thicker than that of the deeper

part of the cell The thick luminal membrane is reported earlier in other areas by Farquhar & Palade (1963) The thicker unit membrane at the luminal surface may imply a difference in rate or nature of exchange of substances across the cell membrane in comparison to that of the intercellular plasma membrane It has not been established whether the thick unit membrane is specific to the marginal cell or to the cells facing the endolymphatic fluid in general, or whether the variation in thickness is due to a substance coating the cell membranes. Further study and technical improvements are necessary before any reasonable conclusion can be made.

The absence of a continuous basement membrane in the stria vascularis of animals is recognized by Iurato (1967) Kikuchi & Hilding (1966) and Takahashi & Kimura (1970) The presence of short segments of the basement membrane is recognized by Hinojosa & Rodriguez Echandia (1966) who believe that they are an extension of the capillary endothelial basement membrane which may serve as a pathway for rapid diffusion of materials from the capillary lumen. The basement membrane is regarded as a barrier to filtrates (Farquhar 1961 Fawcett, 1959) For instance, tracer substances in the absence of a basement membrane spread very rapidly (Zamboni & Pease, 1961) The basement membrane serves also as an attachment for endothelial cells (Stehbens, 1966) Kikuchi & Hilding (1966) report that the basement membrane is evident below the marginal cells in the early developmental phase, but later disappears. The basement membrane seen in the stria could be the remains of developmental changes and possibly serves as a desmosome. Another interesting point is the closeness and frequent association of the basement membrane to the intermediate cells, rather than the marginal and basal cells, which suggests a possibility of the intermediate cells having a stronger functional tie to the basement membrane or secreting a substance to form the basement membrane.

The paucity of desmosomes between the marginal cells and the basal cells is noteworthy They are apparently held together by the interlocking of their cell processes. On the contrary innumerable fasciae and maculae occludentes (Fawcett, 1966) and short desmosomes are outstanding features in the basal cells. The cell junctions between the basal cells and the fibrocytes of the spiral ligament show desmosomes less frequently than those between the basal cells, but more frequently than those between the marginal and the basal cells. Thus the basal cells appear to serve as a supporting platform for attachment of the strial epithelial cells and blood vessels on one side, and of fibrocytes of the spiral ligament on the other and also to provide a rather tight seal separating fluids between the strial epithelia and the spiral ligament.

The blood vessels of the stria vascularis do not differ from those of other areas. Up to the present, neither smooth muscles nor neural elements have been identified. The endothelial cells are not fenestrated and pinocytotic activity is not particularly remarkable. Occasionally they contain bundles of filaments however they are rather longitudinally arranged, local in distribution, and are morphologically unlike the contractile filaments seen in the smooth muscle cells of other areas. They are probably of cytoskeletal nature and may provide elasticity to the cells as proposed by Cecio (1967) The basal cells often contain numerous, short filaments and they encircle the blood vessels, but they are not contractile in type, and are presumed not to take part in active control of vascular flow in or out of the strial layers. Perhaps some of these factors may explain the stable stria vascular flow observed in the living animal by Perlman & Kimura (1955).

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ZUSAMMENFASSUNG

Die Stria vascularis des Menschen enthält drei Zelltypen: marginal, intermediär und basal, die denen kleinerer Tiere sehr ähnlich sind. Die marginalen Zellen haben einen reichlichen Gehalt an homogener elektronendichter Substanz. Sie enthalten auch zahlreiche, umhüllte Vacuolen (coated vesicles), Mitochondrien, granuläres endoplasmatisches Reticulum und freie RNA Körperchen. Die luminare Oberfläche hat oft Microvilli und eine ziemlich dicke Einheitsmembran (unit membrane). Zahlreiche Zellfortsätze dringen tief in die Basis ein, und greifen dabei mit denen der intermediären und basalen Zellen ineinander. Mit Ausnahme der Gegenden, die den Blutgefässen naheliegen, fehlt im allgemein die Basalmembran. Die intermediären Zellen haben ein sehr ausgebildetes Golgi System, agranuläres und granuläres endoplasmatisches Reticulum und auch grosse Mitochondrien. Kurze Abschnitte der Basalmembran sind oft peripheral zu den Zellkörpern gelegen. Die Basalzellen sind lang und flach und haben zahlreiche kurze Desmosome, Fasciae und Maculae occludentes. Die Zellfortsätze werden von ihnen in die anderen strialen Epithelien geschickt dazwischen es aber gibt nur sehr wenige Desmosome. Hier und da findet man Desmosome am Treffpunkt der Basalzellen und Fibrozyten des Ligamentum spirale. Der interzelluläre Raum zwischen den Fibrozyten enthält zahlreiche Fibrillenbündel die im Querschnitt rechtwinkelig sind. Den Blutgefässen der Stria vascularis fehlen glatte Muskelzellen und Nervenelemente außerdem liegen sie eng an den marginalen und intermediären Zellen an.

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ZUM NYSTAGMUSVERHALTEN BEI VORÜBERGEHENDER REIZLOSER AUSSCHALTUNG DER GROSSHIRNRINDE IM BEREICH DES LOBUS TEMPORALIS MITTELS DIREKTER UNTERKÜHLUNG

B Minnigerode

Aus der Hals-Nasen-Ohrenklinik der Universität Göttingen, Göttingen, BRD

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Wiederholte, vorübergehende, reizlose Ausschaltungen der freigelegten Großhirnrinde im Bereich des Lobus temporalis mittels Kälteeinwirkung ergaben einen reproduzierbaren Spontannystagmus zur Seite der Rindenkühlung, der trotz aufrecht erhaltener Ausschaltung maximal nur 2 min 30 sec anhielt. Entstehung und Schwinden des Nystagmus werden als Ausdruck einer vorübergehenden Rechts-Links-Tonusdifferenz beider Vestibularsysteme infolge der Unterbrechung des corticofugalen Steuereffektes auf das cerebellovestibuläre System gedeutet und in Analogie zu dem Verhalten des cardialen Reizleitungssystems beim Versuch von Stannius gesetzt. Auf grund der tierexperimentellen Ergebnisse wird eine Beeinflussung des Vestibularapparates durch die Großhirnrinde als sehr wahrscheinlich angesehen.

heute noch sind die von der Großhirnrinde und vielleicht auch vom Kleinhirn her (Mygind, 1953) kommenden hemmenden Einflüsse problematisch, die tierexperimentell nach Exstirpation einer Großhirnhälfte und auch in klinischen Beobachtungen (De Kleyn & Versteegh, 1927 1928 Vogel 1929 1930 Hallpike *et al* 1951 Hofmann, 1961) zum Auftreten eines richtungsbestimmten Nystagmusüberwiegens zur operierten bzw kranken Seite, anscheinend ohne Spontannystagmus, geführt haben. Wenn es sich — wie man annimmt — in solchen Fällen um die Schädigung gleichseitig hemmender Großhirnbahnen zu den Vestibulariskernen handelt, so wäre bei ihrer Unterbrechung ein Spontanystagmus als Ausdruck einer Vestibularistonusdifferenz zu erwarten (Frenzel, 1955) Es ist schwer vorstellbar daß es corticale, den Tonus hemmende Bahnen zu den Vestibulariskernen gibt, deren Schädigung den experimentellen Nystagmusvorgang im Sinne eines richtungsbestimmten Überwiegens beeinflussen, ohne einen Spontan- oder Provokationsnystagmus zu verursachen. Die folgenden tierexperimentellen Untersuchungen galten daher einer Klärung der Einflüsse corticaler Hemmungsbahnen durch Suche nach dem von Frenzel (1955) postulierten Spontannystagmus im Falle ihrer Unterbrechung

METHODIK

Für das Studium der Funktionen des Zentralnervensystems bergen alle Methoden, die eine Ausschaltung von Zentralteilen durch irreversible Zerstörung zur Grundlage haben, den Nachteil in sich daß sie mit ausreichender Sicherheit keine Rückschlüsse zulassen, ob die beobachteten Symptome wirklich als reine Aufhebung normaler Funktionen oder lediglich als unbeabsichtigte Nebenwirkungen des Eingriffes durch Schädigung oder Reizung benachbarter Hirnteile gedeutet werden können. Ich habe daher zur Klärung der Frage, welche Stellung der Cortex cerebri innerhalb der zentralen Vestibularisbahn einnimmt, in Anlehnung an das Verfahren von Trendelenburg (1910) eine reizlose vorübergehende Ausschaltung der Großhirnrinde mittels direkter Kühlung vorgenom-

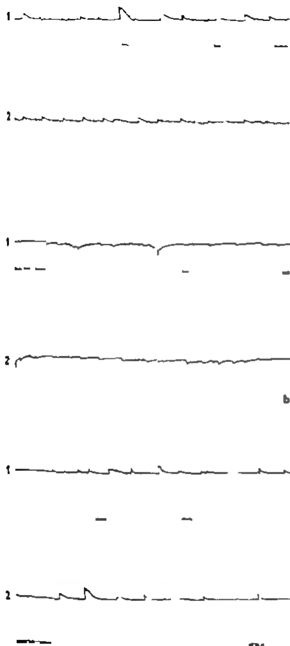


Abb. 1 (a–c) Spontan-Nystagmus nach Großhirnrindenausschaltung im Bereich des Lobus temporalis mittels direkter Unterkühlung; Hebelregistrierung auf Rußpapier unter Verwendung einer schweren Kaninchen-Bulbuskappe mit Trinkhalm-Hebel (modifiziert Kobrak) und Rindenkühlung rechts, *b* Rindenkühlung links; a_1, b_1 = Fortsetzung der Kurven a, b. 1 cm = 10 sec.

men, da ich mir von dieser Untersuchungsmethode bessere Aufschlüsse versprach. Ausgehend von den Untersuchungen Spiegels (1934), der die Rindenfelder des vestibulären Apparates medial von der Hörrinde vermutete, und den Versuchen von Fulton (196?) der bei Strychnisation des Temporallappens Vestibularis-Symptome feststellte, sowie den klinischen Erfahrungen von Fitzgerald & Hallpike (194) habe ich die Abkühlung der Großhirnrinde im Bereich des Lobus temporalis vorgenommen.

Um die Rindenoberfläche des Lobus temporalis der Kühlung aussetzen zu können, wurde nach einem bogenförmigen Hautschnitt im Schläfenbeinbereich eine kreisförmige Trepanationsöffnung von etwa $1\,^1/_2$ cm Durchmesser unter Schonung der Dura angelegt. Durch die Erhaltung der Dura sollte eine Funktionsstörung in der unterliegenden Rinde, besonders infolge Prolabierens der Hirnoberfläche, und ein für die Kühlung störender Blutaustritt aus den Duragefäßen vermieden werden. Die Kühltemperatur wurde mittels einer Kohlensäureschnee-Sonde (Spitzendurchmesser 3 mm) auf −3 bis −5 C eingestellt und dadurch eine Duratemperatur zwischen +11,5 C_{min} und +13,0 C_{max} erzielt. Diese Temperaturwerte reichten zur vollständigen Rindenausschaltung aus, ohne die Rindenstrukturen der Gefahr des Gefrierens auszusetzen. Da auch die plötzlich vorgenommene Abkühlung, sofern dies zur Vermeidung lokaler Reizerscheinungen ohne Druckänderungen geschieht, die Untersuchungstiere unbeeindruckt läßt, konnte sie ohne Betäubung durchgeführt werden. Lediglich der Kopf der sonst ungefesselten Tiere wurde zur schonenden Kälteapplikation und besseren Nystagmusbeobachtung fixiert. Die Ausschaltung konnte zu jeder beliebigen Zeit unterbrochen werden, sie hörte mit Abklingen der Kältewirkung ebenfalls auf. Der einzige Nachteil der Methode lag in der verhältnismäßig kurzen Zeit (höchstens 24 Stunden), die vom Zeitpunkt der ersten Kühlung an für wiederholte Beobachtungen zur Verfügung stand, da sich selbst bei streng aseptischem Verfahren nach etwa einem halben Tage auf der Dura ein Belag bildete,

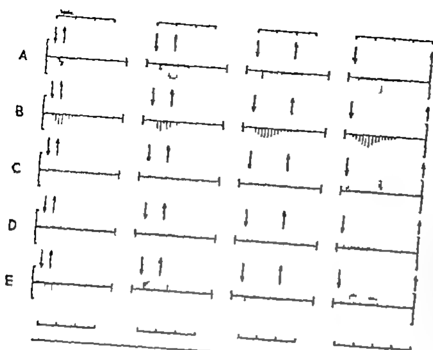


Abb 2 Halbschematische Darstellung des aus Latenzzeit, Dauer Frequenz und Amplitude resultierenden Nystagmusablaufes bei direkter Unterkühlung der Großhirnrinde (Mittelwerte aus jeweils wiederholten Versuchen); *A* und *B* Kühlung im Bereich des linken, *C* und *D* Kühlung im Bereich des rechten Lobus temporalis, *E*, wechselseitige Kühlung.

der den Erfolg der Abkühlung sehr wesentlich herabsetzte

Die Untersuchungen wurden an 5 eben geschlechtsreifen Kaninchen vorgenommen, und zwar bei 2 Tieren durch Abkühlung der freigelegten Rinde über dem rechten Lobus temporalis, bei 2 weiteren Tieren durch Abkühlung der Rinde über dem linken Lobus temporalis und bei einem Tier durch wechselseitige Abkühlung der beiderseits im Schläfenlappenbereich freigelegten Großhirnrinde. Die Rindenausschaltung durch direkte Kälteeinwirkung wurde jeweils über 30 sec, 1 min, 2 min und bis zu 4 min durchgeführt und nach Beendigung der Kühlung das Gehirn sich selbst der Wiedererwärmung überlassen. Die Beobachtung erfolgte bei normaler Kopflage der Tiere unter Lupenvergrößerung und erstreckte sich auf die Entstehung von Spontannystagmus, seine Dauer Frequenz und Amplitude

Untersuchungsergebnisse

Läßt man Kälte über wechselnd lange Zeitdauer auf die Großhirnrinde des Kaninchens im Bereich des Lobus temporalis einwirken, so tritt unter der reizlosen Rindenausschaltung bis maximal 2 min 15 sec — individuelle Schwankungen bis zu ±5 sec unberücksichtigt — er in der Regel wenig frequenter fein bis mittelschlägiger Nystagmus zur Seite der Rindenkühlung hin auf, der mit Beendigung der Abkühlung wieder sistiert. Wird dagegen die Abkühlung bis zu 4 min fortgesetzt, so bricht noch während der aufrecht erhaltenen Rindenausschaltung der Nystagmus nach 2 min 10 sec bis 2 min 30 sec verhältnismäßig plötzlich ab und es tritt wieder Augenruhe ein. Daß es sich hierbei ausschließlich um einen Effekt der Rindenkühlung handelte, zeigte eine einfache thermoelektrische Temperaturmessung, die bei einer Abkühlung der Duraoberfläche auf +11.5 C im Gebiet des Markfaserzusammenschlusses zur inneren Kapsel nur eine Temperaturänderung von 2 C gegenüber der Norm aufwies, die für eine Funktionsaufhebung nicht in Betracht kommen kann. Wurde nach einem Intervall von mindestens 3 Stunden die Großhirnrinde erneut über 1 bis 2 min gekühlt, so ließ sich wiederum der oben beschriebene Nystagmus beobachten. Der gleiche Effekt trat auch bei wechselseitiger Kühlung, jeweils mit Schlagrichtung des Nystagmus zur Seite der Rindenausschaltung hin, ein.

Die während der über 4 min andauernden Rindenkühlung, nach Abklingen des Spontan-

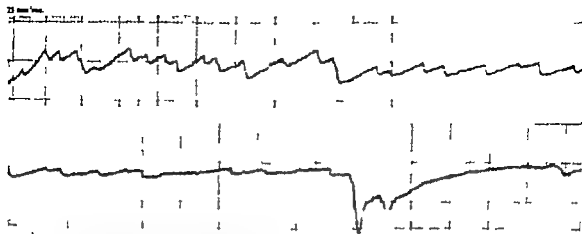


Abb. 3 Ausschnitte aus den bei einem 11-jährigen Kinde mit Calottendefekt über der linken Hemisphäre nach umschriebener Eisapplikation im Bereich des Lobus temporalis gewonnenen elektro-nystagmographischen Kurven.

nystagmus und nach vorausgegangener Abtragung der Ohrmuschel vorgenommene thermische Labyrintherregbarkeitsprüfung durch Gehörgangsspülung mit Wasser von 30 und 44 C für die Dauer von 40 sec ergab hinsichtlich des experimentellen Nystagmus keine verwertbare Seitendifferenz. Ein Nystagmusüberwiegen im Sinne von Fitzgerald & Hallpike (1942) sowie Ino & Takeyama (1961) konnte ich also bei der reizlosen und vorübergehenden Ausschaltung der Großhirnrinde im Bereich des Lobus temporalis nicht feststellen.

In diesem Zusammenhange ist ein Befund von Interesse, den ich bei einem 11 jährigen Mädchen (E C. Poliklin. Nr 4579/63) erheben konnte, bei der zur Exstirpation eines Abscesses in der vorderen Schädelgrube die Calotte über der linken Hemisphäre von der Schläfengrube bis nahe dem Occipitalbereich entfernt worden war Der Knochendefekt war lediglich von der Kopfhaut bedeckt. Die Labyrinthfunktion war bei mehrmaliger Überprüfung beiderseits normal. Nach umschriebener Eisapplikation im Bereich des Lobus temporalis trat nach einer Latenzzeit, die bei wiederholten Prüfungen zwischen 45 und 60 sec schwankte, für die Dauer der Kühlung ein mittelschlägiger nach links gerichteter Nystagmus auf. Wurde die Unterkühlung über … min hinaus fortgesetzt, so wurde die Frequenz und Amplitude des Nystagmus allmählich immer geringer und schließlich von einem Ziehen der Augen in Deviationsstellung nach rechts abgelöst, bis nach etwa 4 min Augenruhe eintrat. Subjektive Mißempfindungen wurden während der Dauer des Nystagmus nicht angegeben. Die Untersuchung erfolgte im Dunkelraum unter der Leuchtbrille in Rückenlage mit um 30 erhobenem Oberkörper und Kopf.

Neuro-physiologische Deutung der Befunde

Zwischen zwei unabhängig voneinander veränderlichen Antrieben können alle nur denkbaren Wechselwirkungen bestehen. Einer kann einseitig einen anderen fördern und antreiben, zwei können einander unterstützen, sie können sich, ohne im übrigen miteinander in Beziehung zu treten, summativ in einer und derselben Verhaltensweise überlagern und sie können einander schließlich wechselseitig hemmen. Es gibt endlich den seltenen Spezialfall, daß von zwei Antrieben der jeweils stärkere den schwächeren in einer nach dem Alles-oder Nichts-Gesetz funktionierenden Kippreaktion ausschaltet. Dabei unterstehen den übergeordneten Systemen häufig mehrere Impulsquellen (sog. Werk-Aktivitäten n K. Lorenz), die aber … zur spontanen Produktion von E…

in der Lage sind. Schaltet man das übergeordnete Zentrum durch einfache Maßnahmen aus, so wird man alsbald gewahr, daß die an das periphere Organ abgehenden Impulse zur Aufrechterhaltung der normalen Funktion vollständig ausreichen. Obwohl also die Werkzeug-Aktivitäten ihre eigene Spontaneität besitzen, werden sie von dem übergeordneten System noch angetrieben, mehr zu leisten, als sie es, sich selbst überlassen, täten.

Ein derartiges Angetrieben-Werden einer an sich spontanen Funktion durch einen von einer übergeordneten Stelle kommenden Reiz ist von den Reizerzeugungszentren des Herzens seit langem bekannt. Der Herzschlag wird normalerweise von den rhythmisch-automatischen Reizen ausgelöst, die der nahe dem Eingang des Blutstromes in den Herzvorhof gelegene Sinusknoten erzeugt. *Etwas weiter Blutstrom-abwärts*, am Übergange in die Kammer befindet sich der Atrio-Ventricularknoten, mit dem Sinusknoten durch ein Bündel reizleitender Muskelfasern verbunden. Beide Knoten produzieren Reizstöße, die imstande sind, die Herzkammer zu einer Kontraktion zu veranlassen. Der Sinusknoten arbeitet schneller als der Atrio-Ventricularknoten. Letzterer kommt daher unter normalen Umständen niemals in die Lage, sich [illegible]tan zu verhalten, denn jedesmal, wenn er [illegible] anschickt, eine Reizsalve abzufeuern, [illegible]ngt ihm der Sinusknoten seinen Arbeitsrhythmus auf. Führt man nun das klassische Experiment von Stannius aus und unterbricht durch Abschnüren des Reizleitungsbündels die Verbindung, so befreit man den Atrio-Ventricularknoten von dem Einfluß des Sinusknotens und der erstere beginnt, nach der sogenannten präautomatischen Pause im Eigenrhythmus Reize zu produzieren.

In einem Verhältnis, das dem des Atrio-Ventricularknotens zum Sinusknoten analog ist, steht nun mit großer Wahrscheinlichkeit auch der Cortex cerebelli im Bereich des Flocculus accessorius zur Großhirnrinde des Lobus temporalis. Durch die Ausschaltung der letzteren würde es demnach zu einer Unterbrechung des *corticofugalen* Hemmungs- oder besser *Steuerungseffektes* auf die nachgeordneten Vestibulariszentren kommen, die eine Störung des tonischen Rechts-Links-Gleichgewichtes beider Vestibularissysteme herbeiführt, deren Ausdruck der von mir beobachtete, zur Seite der Rindenausschaltung hin schlagende Spontannystagmus ist. Seine Dauer wäre der präautomatischen Pause des cardialen Reizleitungssystems gleichzusetzen. Sobald dann das cerebello-vestibuläre System im Eigenrhythmus zu arbeiten beginnt, wird das Tonusgleichgewicht zwischen beiden Seiten wieder hergestellt, wodurch alle Spontanerscheinungen schwinden.

Es muß dabei offen bleiben, ob der Cortex cerebri in einem echten Dominanzverhältnis zum cerebello-vestibulären System steht oder ob zwischen beiden Antriebsquellen eine gegenseitige Beeinflussung im dem Sinne besteht, daß *jede der zwei, Spontanimpulse erzeugenden* Instanzen bestrebt ist, der anderen ihre eigene Frequenz aufzuzwingen und sie in konstanter Phasenbeziehung festzuhalten. Beide Impulsquellen würden dann nach den Gesetzlichkeiten der relativen Koordination (E. v. Holst) arbeiten. Ausschaltung einer Impulsquelle hätte dann zwar stets eine vorübergehende Rhythmusstörung, aber keinen bleibenden Funktionsausfall im Sinne einer Rechts-Links Tonusdifferenz zur Folge. Eine solchte Deutung ließe die Möglichkeit zu, daß durch die Unterkühlung des Cortex nicht die efferenten, sondern unter Umständen isoliert die afferenten Großhirnbahnen blockiert würden.

Die hier dargelegte funktionelle Stellung der Großhirnrinde innerhalb des zentralen Vestibularissystems gewinnt an Wahrscheinlichkeit, da sie sich mit den Untersuchungsergebnissen von Bauer & Leidler (1911), Dusser de Barenne & De Kleyn (1922), Fitzgerald & Hallpike (1942), Crammer (1951) sowie Ino & Takeyama (1961) unter Berücksichtigung der Tatsache, daß die genannten Autoren bei ihren Experimenten irreversible Zerstörungen setzten, ohne weiteres in Einklang bringen läßt. Sie erklärt auch zwanglos, warum Hofmann (1961) nach elektrochirurgischer Abtragung der Großhirnrinde 24 Stunden später bei der Dreherregbarkeitsprü-

fung keine verwertbaren Differenzen im Ablauf des postrotatorischen Nystagmus feststellen konnte. Im Gegensatz zu Hofmann (1961) und in Übereinstimmung mit den oben genannten Autoren glaube ich — mit der bei Tierexperimenten immer gebotenen Zurückhaltung — eine Beeinflussung des Vestibularapparates durch den Cortex als sehr wahrscheinlich annehmen zu dürfen.

SUMMARY

Repeated, temporary non-irritating suspension of the functions of the exposed cerebral cortex in the region of the lobus temporalis by means of cooling processes resulted in a reproducible spontaneous nystagmus on the side where the cortex has been cooled. In spite of the maintained suspension of the functions of the cortex, the nystagmus lasted for a maximum period of only min. The occurrence and disappearance of the nystagmus is taken to be an expression of temporary dextro-sinistral tonus-difference in the two vestibular systems in consequence of the interruption of the corticofugal directional effect on the cerebello-vestibular system, and is rated as analogous to the behaviour of the cardial conduction system in the experiment by Stannius. On the basis of the results of animal experiments, an influencing of the vestibular apparatus by the cerebral cortex is regarded as highly probable.

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OBSERVATIONS ON CENTRAL MECHANISMS COMMON TO VESTIBULAR AND OPTOKINETIC NYSTAGMUS

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The covariation of optokinetic and vestibular nystagmus before and after administration of nicotine was studied. The results indicate that the amplitudes of the vestibular and the optokinetic nystagmus have a common central programming and that the speed of the slow components of the vestibular and the optokinetic nystagmus are influenced by a common central damping mechanism.

The integration of optokinetic and vestibular impulses is of fundamental importance for the regulation of eye movements, e.g. for spatial orientation. The concept of an optovestibular integration was proved essential by the experiments of Jung (1947) who demonstrated that i *(OKN) and vestibular nystagmus* ''N) show algebraic summation. Later *et al.* (1961) claimed that the vestib- and the optokinetic eye movements are programmed in one common system. This was shown by means of vestibular and optokinetic stimulation with slow pendular movements giving the same frequency characteristics. The seat for this integration may reasonably be found in centres common for the optokinetic and the vestibular pathways. Jung & Kornhuber (1964) suggest that the integration of optokinetic and vestibular nystagmus takes place in the paramedian zone of the ponto-mesencephalic reticular formation. Bender & Shanzer (1964) concluded that the oculomotor integration does not occur in one centre only However the ponto-mesencephalic nuclei in the reticular formation were considered an important component for the integration mechanisms.

In contrast to the OKN the subcortical pathways of the VN are fairly well mapped out. It is generally accepted that the slow phase eye speed is the nystagmus quality that most adequately reflects the activity of the peripheral receptor (Henriksson, 1955) From the vestibular nuclei, continuously fed with volleys of electrical activity from the vestibular nerves, the impulses are mediated partly through the *medial longitudinal fasciculus to the ocular* nuclei. Simultaneously the incoming impulses spread to the reticular formation from where the regulation of the rhythmical interruption of the slow components take place (McCabe, 1965) This vestibulo-ocular system is composed of pathways connecting various regions of the cerebrum, brainstem, and cerebellum by means of multisynaptic articulations, and it is influenced by vestibular as well as by visual stimuli (Bender & Shanzer 1964)

The subcortical neuronal responses in optokinetic stimulation are not known in detail. Effects of brainstem lesions on optokinetic nystagmus, however have been investigated by Enoksson (1956) and Teng *et al* (1958) They showed that the OKN can be utilized for localizing the side of the lesion in the posterior cranial fossa. Cohen & Feldman (1968) showed the intimate relation between neuronal activity

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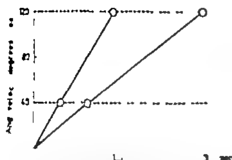


Fig 1 Diagram illustrating the moments (circles) when either the labyrinths or the eyes are equally capable of detecting the velocity of the head. The unbroken lines represent the velocity in degrees per sec of rotary chair in darkness as a function of time. The dotted lines represent the angular velocity difference in degrees per sec between head and surroundings in light as a function of time.

in the brainstem reticular formation and the lateral geniculate body

It is of interest to know how the nystagmus pattern can be influenced by the central structures common to optokinetic and vestibular nystagmus. This knowledge may be obtained as outlined in the following hypothesis.

The retina of the eyes and the receptors of the labyrinths are the two cranial sensory organs that participate in the maintenance of balance. The eyes detect the difference between the velocities of the head and the surroundings. The canals of the labyrinths react on a change of the angular velocity of the head in relation to the surroundings. During acceleration, the canals detect the angular velocity of the head provided that the velocity involved is obtained within 3 sec (van Egmond *et al.* 1949 Henriksson, 1955). In certain conditions, the information of the angular velocity of the head in relation to the surroundings is equivalent irrespective of whether the labyrinths or the eyes have been stimulated (see circles in Fig. 1)

Thus, the CNS will get information about the body in movement from either of two different cranial sensory organs. Furthermore, the optokinetic and the vestibular signals initiate the same kind of motor activity i.e. the nystagmus beats. Therefore, the vestibular and the optokinetic beats should be identical precisely when the supply of the sensory information is of equal magnitude to the two sensory organs. This forms the basis for a study of the relation between the vestibular and the optokinetic nystagmus Since the retina informs continuously on the velocity difference between the head and the surroundings as distinguished from the cupula organs. the nystagmus output would be unchanged during a constant optokinetic stimulation. However all motor activities depend to some extent on central modulation phenomena. Any difference between the initial and the later obtained optokinetic nystagmus may therefore depend on a central processing, provided the peripheral receptivity of the sensory organ remains unaffected.

Any change of the nervous transmission in the common central pathways for the optokinetic and the vestibular reflex arcs should be equally reflected on the respective nystagmus patterns. Thus, from the covariation of the vestibular and the optokinetic nystagmus patterns, conclusions may be drawn about the central influence on the configuration of the nystagmus beat and the nystagmus pattern.

The aim of the present study was to investigate:

1 The normal covariation of the different qualities of the optokinetic and the vestibular nystagmus patterns.

2. The induced covariation obtained by administration of a central acting drug, nicotine (Tibbling, 1969 *a*)

MATERIAL AND METHODS

The material consisted of 12 healthy adults (9 female and 3 male) All smoked at least 10 cigarettes/day All had a smoking-free interval of 12 hours before the test.

Stimulus

The test subject, with head fixed and the lateral semicircular canals oriented in the horizontal plane, sat in a rotary chair The vestibular

stimulation was given by a clockwise angular acceleration of 120 /sec/1 8 sec during 1 8 sec in complete darkness The subject was instructed to keep the eyes open and to report about perception of rotation and non rotation. After a 3 min period of rotation in darkness, the light was switched on. White vertical stripes, 6 wide, placed 120 cm from the test subject and at intervals of 30 on the black surrounding curtains, gave an optokinetic stimulus of 120 /sec. After 20 sec, the light was switched off During the optokinetic stimulation, the test subject was told to work on arithmetic problems to distract conscious attention from the passing stripes. After the light was switched off the registration was continued until the optokinetic afternystagmus disappeared

Nicotine experiment

Five minutes after the vestibular and the optokinetic tests were ended, the subject was told to smoke a cigarette. The smoke was deeply inhaled every 15 sec for 3 min. Immediately after the subject was again rotated as described above, except that the rotation in darkness lasted for only 1 min before the light was switched on Each subject was re-examined in the same manner on two different occasions— week apart—in order to study the reliability he tests.

Recordings

The horizontal eye movements were recorded by means of an electrical DC-technique which utilizes the corneo-retinal potential difference. Before and after each of the separate tests, the horizontal eye movements were calibrated for 50 around the midpoint. The speed of the rotary chair was recorded by means of an optical device. The signals were recorded on a four-channel ink writer (Mingograf 81 Elema Schönander Sweden) fed with a paper speed of 2.5 cm/sec.

Analysis of the recordings

The speed of the slow component of VN OKN and OKAN was calculated at the 2nd, 4th, 10th and 18th second after the appearance of the respective nystagmus. The amplitude of the nystagmus beats was worked out at the same points. The nystagmus frequency per 10 sec was analysed during the first 20 sec of the VN and the OKN and during the first 40 sec of the OKAN The duration of only the OKAN was taken into consideration. The data were statistically evaluated by Student's *t*-test.

RESULTS

Values of speed of the slow component, amplitude frequency and duration refer to the respective mean values of the test group

Speed of slow component

Table 1 gives the mean speed of the slow component of VN OKN and OKAN and the standard error of the means.

The speed of the VN decreased during the first 18 sec of rotation from 60 /sec to 25 /sec. No significant differences were found between the values obtained on the two different test occasions or between the values obtained before and after smoking. *The speed of the OKN was highest at the beginning* of the optokinetical stimulation, 55 /sec, and decreased successively to 42 /sec during the first 18 sec. The corresponding values on the second test occasion (50 /sec and 36 /sec, respectively) did not differ significantly from these obtained on the first test occasion. The speed of the slow components during the first 10 sec after smoking was about 10 /sec lower than that before smoking.

The OKAN was present before smoking in 11 subjects on the first test occasion and in 10 on the second. After smoking, the OKAN appeared in eleven test subjects on both the test occasions. On the first test occasion before smoking, the speed decreased from 18 /sec to 7 /sec during the first 30 sec. The speed was lower on the second occasion than on the first at every point of time studied. The speed of the slow component after smoking was about

Table 1 *Speed of slow component of vestibular (VN), optokinetic (OKN), and optokinetic after-nystagmus (OKAN) before and after smoking*

Mean values (degrees per second) are given with standard error of the mean, = 12. Values in parentheses refer to the second test occasion. The asterisks refer to significance limits of $p<0.05$ ( ), 0.01( ) and 0.001 ( ). The statistical evaluation was performed with the values before smoking as reference

	Time in seconds after onset				
	2	4	10	18	
Before smoking					
VN	69.4 ± 4.4 (65.9 ± 3.4)	53.2 ± 3.7 (58.3 ± 2.6)	37.3 ± 3.6 (38.6 ± 3.1)	25.4 ± 2.6 (24.8 ± 2.1)	
OKN	54.6 ± 3.9 (49.8 ± 5.1)	50.8 ± 5.8 (46.1 ± 5.8)	44.2 ± 4.3 (43.3 ± 5.0)	42.0 ± [illegible]9 (35.5 ± 3.3)	
OKAN	18.4 ± 6.6 = 11 (9.7 ± 1.1)	10.5 ± 2.5 = 11 (6.8 ± 0.8)	5.5 ± 1.4 = 11 (6.4 ± 0.9)	6.6 ± 2.0 10 (4.6 ± 2.9)	6.7 [illegible] 1 9 (4.4 – 2.2) 5
After smoking					
VN	65.5 ± 3.2 (60.9 ± 3.4)	56.3 ± 3.0 (52.3 ± 3.0)	32.7 ± 2.8 (32.7 ± 2.6)	21.3 ± 2.7 (21.5 ± 2.1)	
OKN	44.4 ± 4.2 (40.7 ± 3.9)	39.1 ± 4.6 (32.1 ± 4.4)	37.1 ± 5.2 (32.8 ± 3.8)	35.1 ± 3.7 (37.5 ± 3.4)	
OKAN	12.5 ± 1.8 = 11 (11.1 ± 1.3) = 11	8.8 ± 1.2 = 11 (8.7 ± 1.5) = 11	9.5 ± 1.8 = 11 (7.0 ± 1.1)	12.7 ± 1.9 11 (9.0 ± 1.3)	16.2 ± 1.8 = 11 (13.3 ± 2.2)

Table 2. *Amplitude of vestibular (VN), optokinetic (OKN), and optokinetic after-nystagmus (OKAN) before and after smoking*

Mean values (degrees) are given with standard error of the mean, = 12. Values in parentheses refer to the second test occasion. For explanation of the asterisks, see Table 1

	Time in seconds			
	2	4	10	18
Before smoking				
VN	17.0 ± 2.1 (12.8 ± 1.3)	13.4 ± 1.3 (13.7 ± 1.3)	12.0 ± 1.7 (12.4 ± 1.4)	9.3 ± 0.8 (9.8 ± 1.1)
OKN	14.0 ± 1.5 (15.4 ± 1.2)	13.1 ± 1.7 (9.9 ± 1.0)	11.9 ± 1.5 (12.5 ± 1.5)	13.2 ± 1.0 (11.8 ± 1.2)
OKAN	8.3 ± 1.2 11 (5.8 ± 1.4) = 10	8.4 ± 1.1 = 11 (5.8 ± 1.5) 10	7.2 ± 1.1 11 (5.0 ± 0.9) = 7	11.1 ± 1.4 10 (7.0 ± 2.4) 7
After smoking				
VN	5.9 ± 0.9* (4.8 ± 0.6)	6.0 ± 0.8 (5.3 ± 0.7)	5.6 ± 0.8 (4.2 ± 0.4)	5.2 ± 0.8 (4.5 ± 0.8)
OKN	8.1 ± 1.4 (7.6 ± 1.4)	7.6 ± 1.3 (5.5 ± 0.9)	6.7 ± 1.0* (6.2 ± 0.7)	7.8 ± 0.9 (7.2 ± 1.0)
OKAN	5.5 ± 1.1 11 (3.0 ± 0.5) = 11	4.9 ± 0.9 = 11 (3.5 ± 0.8) 11	4.8 ± 0.7 11 (3.6 ± 0.5)	5.6 ± 1.0* 11 (3.8 ± 0.5)

the same as before smoking during the first 10 sec. Afterwards, however the speed increased slightly so that the values at the 30th second were higher than those at the 2nd second The increase in the speed of OKAN obtained after smoking was statistically highly significant on both test occasions.

Amplitude

Table 2 gives the mean amplitudes of VN OKN and OKAN and the standard error of the means at the different times studied. *The VN amplitude* decreased from 17 to 9 during the first 18 sec. The corresponding values on the second test occasion were 13 and 10 respectively The nystagmus amplitudes during the first 10 sec after smoking were less than half the height of those before smoking. The amplitude decrease by smoking was most pronounced at the beginning of the vestibular stimulation. The mean values did not differ significantly between the two test occasions

The initial *amplitude of the OKN* was of about the same magnitude as the vestibular amplitude. However there was no decrease in the amplitude as long as the optokinetic stimulation lasted. Thus, at the 18th sec, there was significant difference between the amplitude vestibular and of optokinetic origin. The amplitude was not significantly changed repetition of the tests. After the smoking procedure, the amplitude decreased in relation to that before by about 5 at each point of time measured after the start of stimulation (Table 2 and Fig. 2) The decrease in the amplitudes on the two different test occasions was statistically significant.

The amplitudes of the OKAN were almost half those of the VN and the OKN The amplitudes were unaffected as long as the OKAN persisted. They were throughout lower on the second test occasion, however although this difference between the two test occasions was not statistically significant. Further reduction of the amplitudes appeared after smoking on both the test occasions.

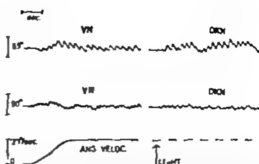


Fig 2 Reproduction of the initial nystagmus patterns obtained by vestibular and optokinetic stimulations before smoking (upper recording) and after (lower recording).

Frequency

Table 3 gives the mean nystagmus frequency per 10 sec of VN OKN and OKAN and the standard error of the means *The frequency of the VN* during the 1–10 and 11–20 sec periods of rotation were slightly lower than the corresponding frequencies obtained on the second test occasion (26.3 to 28 7 and 21 4 to 24 1 beats) After smoking a cigarette the nystagmus frequencies were almost doubled. The increases were statistically highly significant.

The frequency of the OKN was very similar to that of the VN during the first 10 sec. After smoking, the frequency was increased about 7 beats per 10 sec (28 to 35 beats) *The frequency of the OKAN* was calculated in periods of 10 sec during the first 40 sec. As Table 3 shows, the values before smoking were low in relation to both VN and OKN and rather constant during the four 10-sec periods" After smoking, the nystagmus frequency on both the test occasions was more than twice that before smoking. The nystagmus frequency increase reached its maximum during the "21–30 sec period" after onset of the OKAN The fluctuation of the frequency maximum was consistent with the fluctuation of the speed of the slow components of the OKAN

Duration

The mean values of the duration of OKAN were calculated with a maximum duration of

Table 3. *Frequency per 10 sec of vestibular (VN) optokinetic (OKN) and optokinetic after-nystagmus (OKAN) before and after smoking*

Mean values (beats per 10 sec) are given with standard error of the mean, [illegible] = 12 Values [illegible] refer to the second test occasion. For explanation of the asterisks, see Table 1

	Seconds			
	0–10	11–20	21–30	31–40
Before smoking				
VN	26.3 ± 1.9 (28.7 ± [illegible].0)	21.4 ± 1.6 (24.1 ± 1.8)		
OKN	27.5 ± 1.7 (25.7 ± 2.2)	26.1 ± 1.6 (25.4 ± 1.7)		
OKAN	9.8 ± 1.2 [illegible] = 11 (10.1 ± 1.4)	8.4 ± 1.1 *n* = 8 (9.7 ± 0.8)	9.6 ± 1.3 9 (7.9 ± 1.5) = 7	8.9 ± 1.5 8 (8.0 ± 2.7) = 4
After smoking				
VN	48.8 ± 3.9 (51.4 ± 3.2)	38.6 ± 3.2 = 11 (38.7 ± 3.1)		
OKN	34.6 ± 3.3 *n* = 11 (34.8 ± 2.4)	31.6 ± 2.6 11 (31.3 ± 1.6)		
OKAN	19.8 ± 2.7 = 10 (20.0 ± 2.2) = 11	21.8 ± 2.4 11 (20.0 ± 2.2)	23.3 ± 2.4 (24.9 ± 2.3)*	23.8 ± 2.1 11 (21.3 ± 2.8)

120 sec in each subject, although after smoking, the OKAN lasted longer in some of the subjects. In the smoking-free tests, the mean duration was 41 ± 7.6 sec (± S.E.M., $n = 11$) on the first occasion and 22 ± 4.9 ($n = 10$) on the second. After smoking, the corresponding values were 115 ± 20.5 sec ($n = 11$) and 98 ± 11 sec ($n = 12$), i.e. the duration was decreased by repetition and increased after the smoking test.

DISCUSSION

To enable us to compare the activity of the vestibular and the optokinetic reflex arcs, signals from regions other than the respective sensory organs must be reduced to a minimum. The VN was therefore provoked in complete darkness. The eyes were open to prevent a conceivable vertical upward movement of the eyes by eye closure, which might change the horizontal eye movements. Moreover open eyes prevent a decrease in vestibular activity (Guedry & Montague, 1961). The degree of alertness, important for the VN intensity (Collins & Guedry 1961) was held as constant as possible by mental tasks, i.e. attention to the sensation of rotation

The optokinetic nystagmus, which also varies with attention (Jung & Kornhuber 1964) or with conscious tracking, was provoked during deprivation of ocular attention. This was done by instructing the test person to do mental arithmetic problems as rapidly as possible. As suggested by Collins & Guedry (1961) such arithmetic tasks ascertain the control of the arousal level and counteract the decline of nystagmus intensity by decreasing alertness. A possible stronger mental activity in the OKN tests than in the VN tests, giving a more intensive vestibular nerve activity must be taken into account by evaluation of the present results.

Speed of slow component

The purpose of OKN as well as VN is to track a moving object. This reflexogenic tracking by vestibular stimulation is to some extent inadequate since the eye speed of the slow component lags behind the stimulus vel[illegible] as was shown by Henriksson (1955)

ging eye speed behind the stimulus velocity increases with increasing age (Tibbling, *1969 b*) From the present results, it is obvious that also the speed of the slow component of the OKN which depends on the velocity difference between the head and the surroundings and not on the number of stripes (Honrubia *et al.*, 1967) lags behind the stimulus velocity A comparison of the initial speed of the slow component of OKN and VN when the two sorts of stimulation are equivalent (see introduction and Fig. 1) reveals that the magnitude of the speed lag is about the same in OKN and VN The lagging eye speed behind the stimulus velocity common to the initial OKN and the initial VN indicates a common central regulation mechanism for the nystagmus eye speed

Regarding the optokinetic eye speed of the slow component as a function of time, the present results show a slight exponential slope of the speed (see Fig. 3) This argues in favour of a central damping mechanism since the peripheral stimulation was constant. The damping mechanism resembles the mechanism applicable to the movements of a damped pendulum. It seems reasonable that the postacceleratory slope of the vestibular slow phase eye speed is also to some extent damped from the same centres as the OKN and is not merely a function of the cupula deviation. Based on experimental work, similar thoughts have been discussed by Collins & Guedry (1961) and by Fluur & Mendel (1969)

The vestibularly induced eye speed of the slow component after smoking remained unchanged this confirms earlier results by Tibbling & Henriksson (1968) and Tibbling (1969 *a*) In the present study the optokinetically induced eye speed of the slow component was lower after the smoking test. This decrease is probably due to the design of the test: in order to investigate the effect of smoking as soon as possible after the test person had stopped smoking, the optokinetic nystagmus was provoked within 1 min postacceleratorily At that time, the vestibular postpost-nystagmus,

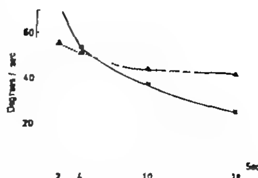


Fig 3 Speed of the slow component of vestibular and optokinetic nystagmus as a function of time. Vestibular stimulus = 120 /sec/1.8 sec during 1.8 sec. Optokinetic stimulus = 120 /sec. ——— = VN (mean values, *n* = 12). Δ — — Δ = OKN (mean values, *n* = 12). The mean values refer to the first test occasion.

directed opposite to the induced optokinetic nystagmus, was continuing and in many cases was even at its maximum. This mode of action made the speed values of the OKN equal to the presmoking values of the OKN minus the speed of the postpost-nystagmus. It is therefore suggested that smoking does not affect the eye speed of the optokinetically induced slow component, any more than it affects the vestibular slow component To prove this definitively further experiments with an optokinetic drum are needed.

Amplitude

The nystagmus amplitude is here defined as a quality of the fast component since the amplitude is an expression for when the slow component is interrupted by the fast one (Tibbling, 1969 *b*)

In the present study the amplitude was about the same height in the initial vestibular and the optokinetic nystagmus beats. During the course of the test, however the optokinetic nystagmus amplitude was mainly unchanged despite the decreasing speed of the slow component. This was in contrast to the decrease found in the vestibular nystagmus amplitude by decreasing speed of the slow component. It seems reasonable to conclude that the amplitude depends on peripheral as well as central factors, and that only strong changes in the sensory input are reflected in the amplitudes.

This argument is further supported by the relations obtained between the amplitude and the speed of the slow component of the OKAN as a function of time. The amplitude of the OKAN was about half that of the vestibular and the optokinetic amplitudes, and at the same time, the speed of the slow components was one-fourth. The decrease in the slow eye speed of the OKAN to half that of the initial value was insufficient to bring about a decrease in the amplitude of OKAN

The smoking-induced decrease in the vestibular amplitudes, reported in an earlier publication (Tibbling & Henriksson, 1968) was confirmed. The present study has demonstrated that smoking also provoked a decrease in the amplitudes of the OKN and the OKAN Indeed, the amplitude decrease in the OKN and the OKAN was not so pronounced as that of the VN Since the nicotine in the cerebrum is rapidly eliminated (Schmiterlöw *et al.*, 1967 Tibbling & Henriksson, 1968) the reduced influence on the OKN and the OKAN amplitudes in relation to the vestibular amplitudes could be explained by the longer time interval that elapsed between the smoking and the generated OKN and OKAN than that between the smoking and the generated VN

The influence of smoking on the amplitudes of VN OKN and OKAN seems to be of central origin since the sensory stimulations and the speed of the slow component, reflecting the strength of the stimulations, were mainly unchanged. This central influence possibly emanates from the structures in the reticular formation that programme the fast phase (Mc Cabe, 1965) The covariation of the amplitudes of VN OKN and OKAN argues in favour of a common centre for the fast components The results support the hypothesis by Trincker *et al.* (1961) about a programming system common to OKN and VN

Frequency

The striking conformity of the initial vestibular and optokinetic nystagmus frequency found in the present work agrees with the results by Trincker *et al* (1961) In a study of the nystagmus frequency it must be observed that the frequency depends on the nystagmus amplitude as well as on the speed of the slow component. Thus the nicotine induced increase in the frequency of the VN is due to a decrease in the amplitudes, whereas the nicotine induced increase in the frequency of the OKAN is due to both an amplitude decrease and an increase in the speed of the slow components Finally the less pronounced frequency increase in the OKN in relation to VN induced by smoking is due to the decrease in the speed of the slow component which counteracts the frequency increase obtained by lowered amplitudes.

OKAN

When dicussing the OKAN it must be observed that this nystagmus is not a secondary nystagmus of conventional type, i.e. a nystagmus of opposite direction to the initial nystagmus. Rather it must be compared to a vestibular nystagmus appearing when the stimulation disappears. After the smoking test, however the OKAN differed in many ways from the VN The nystagmus intensity of the OKAN increased after 18 sec, giving an increasing speed in the slow component and indirectly an increase in the nystagmus frequency Furthermore, the duration of OKAN was prolonged by more than three times the presmoking values Whether this increase in the OKAN intensity obtained after smoking is due to a vestibular after-postpostnystagmus interfering with the OKAN or to some sort of central release mechanism is at the moment unclear

CONCLUSIONS

1 The optokinetic induced speed of the slow nystagmus component is lower than the speed of the optokinetic stimulus, provided no voluntary tracking is involved This relation between the stimulus and the resulting eye speed in adults is of the same magnitude as that between a corresponding vestibular stimulus and the resulting vestibular eye speed.

2. The initial amplitudes of the vestibular and the optokinetic nystagmus beats are of the same height when the respective sensory organs are stimulated equally

3 The initial nystagmus frequencies obtained by a vestibular or an optokinetic stimulus of analogous power are equal.

4 During a constant optokinetic stimulation, the optokinetic nystagmus shows a decrease in the speed of the slow components and in the frequency similar to but less pronounced than the postrotatory vestibular nystagmus.

5 The speed of the slow component of the vestibular and the optokinetic nystagmus is mainly unchanged by smoking.

6 After smoking, there is a clear-cut decrease of the amplitudes of the vestibular and optokinetic nystagmus and the optokinetic afternystagmus.

7 After smoking, the duration of the optokinetic afternystagmus is markedly prolonged.

8. The results support the hypothesis of a common system within the CNS that programmes the vestibular and the optokinetic nystagmus, and to some extent also the optokinetic afternystagmus.

ZUSAMMENFASSUNG

Zusammenhang von optokinetischem und vesti- Nystagmus wurde vor und nach der Ver von Nikotin studiert. Die Ergebnisse deuten daraufhin, dass die Amplituden des vestibulären und des optokinetischen Nystagmus von einer gemeinsamen Zentrale usprogrammiert werden und dass die Geschwindigkeit der langsamen Phase des vestibulären und des optokinetischen Nystagmus durch einen gemeinsamen zentralen Dämpfungsfaktor beeinflusst werden.

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ON VESTIBULAR RESPONSE TO MECHANICAL IRRITATI

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In the belief that stapedectomy provides an opportunity for studying labyrinthine response to mechanical irritation, peroperatory electronystagmography was performed on 10 consecutive otosclerotic patients. The results of the investigation show that mechanical irritation, such as produced during stapedectomy has no stimulating effect upon the vestibule, recordable by means of electronystagmography

The bulk of our present day knowledge regarding vestibular functions in the human is based on either caloric irritation of the end-organ (one at a time), or on various rotatory tests which stimulate both labyrinths simultaneously In the belief that middle ear surgery provides an opportunity for studying labyrinthine responses, the authors had previously performed *post-operative electronystagmographic* studies in 284 consecutive cases. This did not reveal a definite and conclusive relationship between irritation of the inner-ear and nystagmic response (Man & Leventon, 1969)

Considering that peroperative electronystagmographic recordings at different stages of stapedectomy may provide a better opportunity to elucidate labyrinthine response to mechanical irritation, such a *study* was undertaken.

METHOD

Ten consecutive otosclerotic patients were the subjects of this investigation. The operation was performed uniformly via the transtympanic route, under local anesthesia (1% Procaine) with no premedication. A fascia-wire prosthesis was used. None of the cases requi a drill-out procedure.

ENG electrodes were fixed and remained in place throughout the surgery A recording was performed prior to as well as at the following stages of the operation 1 Elevation of the tympanic flap 2. Removal of the posterior bony canal wall 3 Extirpation of the foot plate 4 Insertion of the prosthesis. Additional ENG tracings were done on the second post operative day

RESULTS

In 8 out of the 10 cases no nystagmus appeared at all In 1 case there were nystagmus beats after insertion of the prosthesis. They were in the direction opposite to the operated ear In the remaining case there were some nystagmic waves after removal of the posterior bony canal wall *The direction there*, was t wards the operated ear

The ENG tracings on the second post op erative day demonstrated nystagmus in all cases In 7 patients it was directed towards the operated ear in 2 away from it and in 1 case it was a direction-changing nystagmus

DISCUSSION

Even though some authors tried t recor labyrinthine respon during surgical ir tion, this was done mainly when oth

radiation was employed. To the best of our knowledge only Majoros (1967) used nystagmography during stapedectomy but this was in order to establish when cochlear damage had occurred. Nevertheless, his findings regarding the lack of nystagmus were similar to ours

Arslan (1964) concluded that nystagmus occurring during the ultrasonic irradiation of the labyrinth was probably due to thermic irritation Stahle & Sahl (1964) believe that this was due to the ultrasonic energy itself Although these authors differ on the subject of etiology on this nystagmus, they do not consider the factor of mechanical irritation.

The post-operative nystagmus which appeared in all our cases is probably due to a post operative labyrinthine reaction, and not to mechanical irritation during actual surgery Shea (1963) and Shambaugh (1963 1967) already suggested that post-operative nystagmus is due to a labyrinthine reaction (serous labyrinthitis) which occurs some time after surgery the time interval being as yet undefined

The results obtained demonstrate that mechanical irritation, such as produced during stapedectomy has no stimulating effect upon the vestibule which can be measured and/or by means of ENG

ZUSAMMENFASSUNG

In der Annahme dass die Stapedectomie Gelegenheit zum Studium der Reaktion des Labyrinths auf mechanische Irritation bietet, wurde an 10 otosklerotischen Patienten eine per-operative Elektronystagmographie durchgeführt.

Die Elektronystagmographie hat gezeigt, dass die während einer erzeugten Stapedectomie mechanische Irritation anscheinend keinen stimulierenden Effekt auf das Labyrinth hat.

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ELECTROGUSTOMETRY

Spatial Threshold Variations

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The distribution of the fungiform papillae f the tongue is known to be uneven. In order to demonstrate similar spatial variations of the sensitivity to electric taste stimulation, thresholds were registered in 10 normal subjects at six different points on the tongue surface. The sensitivity was found to be greatest along the edge of the tongue, usually with minimum threshold at the tip of the tongue. The individual differences were rather pronounced and moderate threshold differences between symmetrical points on the two sides were found in few cases. As the stimulating electrode cannot be applied repeatedly to exactly the same point, the spatial threshold variations may influence the results f clinical taste examinations. I order to ensure selective stimulation f the two sides, point on the edge of the tongue, 1 / cm from the midline is usually preferred for clinical threshold determinations. The threshold at this point was found to be representative and close to the minimum threshold in all subjects.

In a previous publication (Føns & Osterhammel, 1966) it has been demonstrated that the subjective intensity of an electric taste stimulus depends on the size of the stimulated tongue area. The findings of v Skramlik (1926) offer a good explanation of this fact. Using chemical taste solutions carefully applied with a pipette, he showed that the taste sensation is proportional to the number of stimulated fungiform papillae.

From a *clinical and experimental point of view* an even distribution of the fungiform papillae over the whole tongue surface would therefore have been ideal. v Skramlik (1924) found the actual distribution rather far from this ideal. A very high density of papillae on the tip of the tongue a gradual decrease backwards along the edge and an extreme scarcity on the mid dorsum were typical findings. Symmetrical areas on the two sides were often unequally covered with papillae In relation to clinical electrogustometry this means that a 5 mm, circular metal electrode may cover a varying *number of papillae depending on the* site of application. As the stimulating electrode cannot be applied repeatedly to exactly the same point on the tongue surface the threshold determinations may vary accordingly If the threshold at the tip of the tongue is considerably lower than at the part of the tongue edge usually preferred for clinical taste examinations, the latter point seems less representative and not quite suitable for the demonstration of ageusia. In view of these implications, it was decided to investigate the spatial threshold variations experimentally

METHOD

Six flat, circular steel electrodes, 5 mm in diameter were embedded in the inside wall of the tongue holder shown in Fig. 1 The wing-shaped lateral extensions were held between the *molar teeth, while the cone-shaped tip* in front contained the flexes. Electrode 1 corresponds to the mid-dorsum electrode 4 to the tip of the tongue. Electrodes 3 and 5 symmetrical points on the edge of

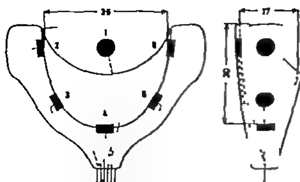


Fig 1 The acrylic tongue holder in which the stimulating electrodes were embedded.

$1\,^1/_2$ cm from the midline approximately off the canine teeth. These are the two points recommended by Krarup (1958) for clinical taste examinations. Electrode 2 and 6 are placed symmetrically farther back on the edge of the tongue Thus, only the anterior two-thirds of the tongue, innervated by the chorda tympani on the two sides, were examined. The subjects were instructed to keep the tip of the tongue in contact with electrode 4 and as far as possible avoid movements of the tongue during the trials.

As stimulator was used a transistorized d.c. stant current generator The stimulus inlty was controlled by means of two wire und potentiometers mounted on the same axle and connected so that the current varied with the square of the angle of rotation. A gradual turn of the knob thus gave an exponential change of the stimulus intensity which is in good accordance with the physiological variations of the taste sensitivity

A number of rectangular stimuli, 500 msec in duration were presented with 4 sec interval. The stimulating impulses were followed by the *audible clicks* of the relays, thus characterizing the method as a so-called "forced choice procedure" According to the "method of adjustment" the subject controlled the stimulus intensity himself by turning the knob of the potentiometer Alternately increasing and decreasing the stimulus intensity he found the lowest detectable intensity which was then registered by the examiner

Ten subjects, 5 male and 5 female all 20-25 years old, went through five trials each. One trial lasted about 45 min and consisted of four threshold *determinations with each of* the six electrodes. This gave a total of 1200 threshold determinations, 120 in each subject, 200 with each electrode.

RESULTS

Fig. 2 and Table 1 give the average thresholds and corresponding standard deviations for the six electrodes. The results were registered in µA and converted to Krarup's logarithmic Electric Gust Units (1958) before the calculation of mean values and standard deviations. The final results were then reconverted to µA, but the diagram in Fig. 2 has a logarithmic ordinate

As expected, the lowest average threshold was found on the tip of the tongue (electrode 4) whereas the threshold increased slightly backwards along the edge of the tongue (electrodes 2, 3 5 and 6) For these five electrodes, however all average thresholds lie within the rather narrow range of 2 0–6 1 µA, a spread which is very moderate compared with the average standard deviations and the total range of the gustometer (0–300 µA) These average

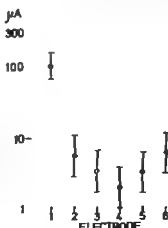


Fig 2 Average thresholds and corresponding standard deviations for the six electrodes.

Table 1 *Average thresholds and standard deviations obtained with the six electrodes*

Electrode	Average threshold	Limits
1	100.0	168.0 64.0
2	5.5	10.8 2.8
3	3.4	6.5 1.7
4	2.0	4.0 1.5
5	3.3	6.4 1.7
6	6.1	11.7 3.2

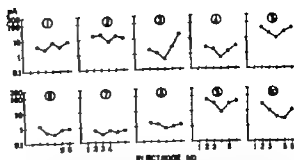


Fig. 3 Graphic representation of the individual results. The threshold points of electrodes 2–6 were connected by solid line which corresponds to the tongue seen from the front.

threshold showed no difference between the right and left side. Electrode 6, on the other hand, gave an average threshold which was considerably higher than for any of the other five electrodes. Fig. 2 also serves to demonstrate that the standard deviation depends on the level of intensity. In this semi-logarithmic diagram the standard deviation is lowest for electrode 6 and highest for electrode 4 which is in good accordance with the discrimination ability (Føns & Osterhammel, 1968).

Fig. 3 gives the individual results for each of the 10 subjects. The points representing the thresholds at electrodes 2–6 were connected by a solid line which corresponds to the tongue holder seen from above or the stretched out tongue seen from the front. The most striking feature is the pronounced individual differences. Although most of the subjects have the lowest threshold on the tip of the tongue, subjects 1, 7 and 10 are exceptions from this. All the subjects had the highest threshold on the mid-dorsum but it varied within the broad range of 26 μA (subject 7) to over 300 μA (subjects 5 and 8). The side differences were usually small, but subjects 3 and 10 showed considerable asymmetry (cf. Table 2).

Table 2. *The difference between the right and left side of the tongue was often considerable; the thresholds determined with electrodes 3 and 5 were therefore compared statistically*

Subject	Difference right–left (EGU)	P-value
1	4.10*	0.05–0.025
2	1.65	0.10–0.05
3	8.20*	0.01–0.005
4	3.55*	0.025–0.02
5	1.10	0.40–0.30
6	3.50*	0.20–0.10
7	3.15*	0.05–0.025
8	3.25*	0.025–0.02
9	1.50	0.50–0.40
10	11.25*	0.001

Difference greater than 2 EGU
Statistically significant difference

The accuracy with which the subjects reproduced their thresholds appears from Table 3. Here again, the individual differences are pronounced. The values are given in Electric Gust Units in order to minimize the influence of the stimulus level.

DISCUSSION

The present findings provide a [illegible] equivalent to v. [illegible] morphology [illegible] of the taste organ. They describe the [illegible] taste on the anterior two-thirds of [illegible] as being roughly limited to a [illegible] around the edge of the tongue [illegible] an average over subjects [illegible] spatial threshold variations [illegible] variations may be considera[illegible]

Table 3 *Average standard deviation given in Electric Gust Units for each of 10 subjects*

Subject	Average standard deviation (EGU)
1	5 72
2	3.20
3	7 60
4	5 04
5	4.55
6	8.39
7	4.65
8	4.01
9	7.50
10	7.30

subjects. Nevertheless, the threshold on the tip of the tongue is not generally so much lower than at the sites of electrodes 3 and 5 that the latter points cannot be considered suitable for the demonstration of ageusia by means of a standardized procedure

In wiew of the spatial threshold variations, the accuracy of the electrode application deserves great attention. It has already been mentioned that under clinical circumstances, the electrode cannot be applied a number of times to exactly the same spot on the tongue surface. Undoubtedly this accounts for part of the un- y of clinical threshold determinations sprte of this, the control of the site and extension of the stimulated tongue area is much easier with electric than with chemical stimulation. Consequently the spatial threshold variations provide another reason for preferring electrogustometry for clinical purposes.

The question of threshold differences between the two sides concerns electrodes 3 and 5 in particular as these were placed on the sites selected by Krarup for clinical electrogustometry In 140 normal subjects Krarup found that in all age groups the threshold varied within a wide range, while the difference between the two sides was equal to—or less than—2 EGU in 99% of the subjects. As seen from Table 2 only subjects 2, 5 and 9 of the present study fall within this 2 EGU range The side difference, however was only statically significant in subjects 3 and 10 The apparent discrepancy between the findings in the two studies should be taken with some reservation as the methods employed were quite different. The present material of 10 subjects is small compared with Krarup's of 140 subjects. On the other hand, the 10 subjects, chosen at random and found to be normal from Krarup's criteria, were tested repeatedly with the very reliable "method of adjustment" In view of the varying profiles of the individual curves in Fig. 3 and the fact that none of the thresholds were remarkably high, it seems likely that subjects 3 and 5 represent extreme variants within a normal range.

In conclusion it can be stated that a rather uneven distribution of the sensitivity over the tongue surface seems quite consistent with normal afferent taste pathways If clinical electrogustometry shows a moderate threshold difference between symmetrical points on the two sides, repeated examinations over a period of time should be carried out and the results be interpreted as part of the complete otoneurological picture of the case.

ACKNOWLEDGMENT

The author is indebted to P A. Osterhammel, B.Sc., and O Arndal for the manufacturing of the electronic equipment.

ZUSAMMENFASSUNG

Es ist wohlbekannt, dass die Verteilung der Papille fungiformes der Zunge uneben ist. Die entsprechende Variation der Geschmacksempfindlichkeit wurde in 10 Versuchspersonen auf 6 verschiedene Stellen der Zungenoberfläche festgestellt. Die Schwellenwerte der Zungenspitze waren durchwegs geringer als auf den Ränder aber beträchtliche individuelle Unterschiede und mässige Unterschiede zwischen symmetrischen Stellen wurden gefunden. Die Zungenmitte wies keines oder nur geringes Geschmacksvermögen auf. Diese örtliche Variation der Empfindlichkeit kann wahrscheinlich die klinische Schwellenbestimmung beeinflussen. Der Schwellenwert auf dem Zungenrand 1 / cm von der Mittellinie war in den meisten Fällen repräsentativ

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The William and Harriet Gould Foundation announces the recipient of the 1969 Gould Award: Dr Eva Sedlackova of Praha, Czechoslovakia. Dr Sedlackova was honored for her research on the development of voice in infants and children.

The Gould Award is presented annually for outstanding fundamental contributions in the field of laryngology. The recipient is selected by an International Committee which at present consists of Dr Renato Segre, Rodriguez Pena 1675 Buenos Aires, Argentina, Professor Dr h.c. Michel Arslan, University of Padua, Padova, Italy Professor Dr Mil Seeman, Nabrezi Bedr Engelse 66, Praha 2, Czechoslovakia, and Dr Hans von Leden, Institute of Laryngology and Voice Disorders, Los Angeles, California, Chairman.

The Award consists of an illuminated plaque and cash prize. Nominations for the 1970 Award should be filed with one of the Committee Members as soon as possible.

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CERVICAL CHORDOMA

Report of a Case and a Brief Review of the Literature

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A case of cervical chordoma in a 54-year-old woman is reported. This case was characterized by the site of the tumour the symptom-free course up to the terminal phase, and the cause of death which was massive subdural and subarachnoid haemorrhage throughout the length of the spinal cord due to invasion of the vertebral artery Perusal of the literature has not revealed symptoms which are pathognomonic, or even characteristic, of chordomas, neither of cranial, cervical nor sacral situation. The course differs according to the site, the survival time for patients with cranial and cervical chordomas be ing ~4 years, while for patients with sacral tumours it averages 5–7 years. The characteristic histology the only means of making an exact diagnosis, is described. In addition, it was attempted to character ize the tumour histochemically by various mucus stainings. This showed that the ground substance consists of acid mucopolysaccharides.

Chordomas are tumours arising from remnants of the notochord, whether it is in a normal or ectopic situation. These tumours are comparatively rare, and there are no cases on record in which the cause of death has been invasion of the vertebral artery leading to massive subdural and subarachnoid haemorrhage. Such a case will be reported below It is further remarkable because of the paucity of symptoms and by its situation in the cervical spine.

Case Report

A 54-year-old woman with history f operations for benign breast tumour and for otitis med Dur ing the month prior to her present admission, on 4.3 1952, she had been aware of a nodule on the posterior pharyngeal wall. It had grown somewhat, but had not given rise to subjective discomfort. Physical examination revealed a tumour the size of a hazel nut, just medial to the lateral retropharyngeal lymph nodes on a level with the soft palate. It was firm, elastic, nodular fixed, and covered with normal mucous membrane No regional lymph nodes were palpable. X-rays of the cervical spine showed moderate spondylosis. X-rays of the chest revealed a couple of ill-defined infiltrations in the 4th and 5th ribs on the left and pulmonary emphysema. Hb 80%, ESR 6 mm, B.P 150/90, weight [illegible] kg. At operation the tumour was found to extend towards the base of the skull and to be quite firmly adherent to the 2nd and 3rd cervical vertebrae from which it had to be scraped with a sharp spoon, while it could fairly easily be detached inferiorly In addition, the two affected ribs were resected. Histological examination of the removed tumour tissue revealed chordoma, and microscopic examination of the ribs showed the sequelae of rib fractures. Thereafter the patient stated that about 2 / years previously she had sustained a trauma to the left chest with typical fracture pain. The postoperative course was uneventful, and X-ray therapy total dose 1,500 R, was administered to two fields on the neck.

The patient was re-admitted 7 months later. In the meantime there had been no complaints. Physical examination showed the appearances to be exactly as at the first admission. The X-ray appearances f the cervical column were unchanged, and operation revealed the mucous membrane and surroundings to be free of tumour At the bottom of a deep crater in the second cervical vertebra there was spongy bone. Postoperative irradiation, 1,500 R.

Six months later the patient complained of rheumatism at the back of her head and neck, radiating to both shoulders. The pain was elicited by head movements, by cool weather and by draught. Still no throat complaints. Radiography of the cervical spine showed a pea-sized translucency anteriorly and to the left of the midline in the second cervical

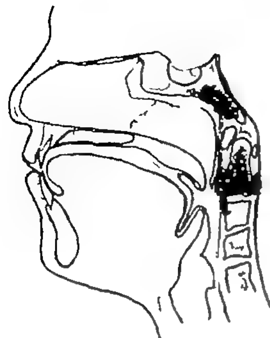


Fig 1 Extent of tumour

vertebra. At operation the tumour was found to have progressed, spreading like a cord upwards and downwards, with a submucous outrider to the right. The cord was removed, in healthy tissue it was believed, but the actual tumour was lying like sago gruel in the previous operative cavity Again 1,500 R was administered, but repeated X-ray examination of the cervical spine showed further destruction at the root of the second cervical vertebra, while the articulation between the 1st and 2nd vertebrae was still uninvolved.

Three months later the patient reported that she had been feeling well, apart from the last 10 days. In the act of defaecation she had developed violent headache over the vertex, and during the subsequent days the pain alternated between the head and spine. No other complaints. Shortly after her admission, however, the patient collapsed when she was going to sit up in bed. She developed clonic twitchings, woke up for brief moment, but when she again wanted to sit up she had another seizure, lost consciousness, and died couple of days later

Autopsy (extract)

The clivus Blumenbachii was chiselled, but no gross abnormality came to light. At the base of the brain, especially around the pons, medulla oblongata and cerebellum there were widespread, blackish-red haemorrhages p to 6 mm thick, which in places had broken through the dura but were predominantly subarachnoid. The body of the second cervical vertebra had been almost completely destroyed by mucinous tumour th the prese h l vesicles 1 3 mm in d this ertebra appeared proper measured ab invasion of the spina corresponding exactl artery The dura was revealed massive su d r orrhage—5 mm thick—t spinal cord. Otherwise th remarkable—especially no

Historical

Virchow (1857) is often ited discovered the chordoma, b he did not notice this type of Luschka (1856) had described so gelatinous neoplastic masses on Blum nb clivus. Virchow believing that they w d rived from degenerated cartilaginous tis ue called them ecchondrosis physaliphora. Mu (1858) demonstrated the similarity to chordal tissue, and this was confirmed by Ribbert s ex periments (1894) He punctured the nucleus pulposus of rabbits. The tissue which escaped from this hernia grew into a greyish-red tumour of the same histological architecture which had been described previously Ribbert was the first to employ the term chordoma. His experiments on rabbits have later been repeated by Congdon (1952) with the same re sult. Stewart & Morin (1926) have used the term "ecchordosis physaliphora" of the chor dal tissue which some authors maintain is present on the clivus in 1 $/_2$–2% of all autopsies (Ribbert, 1894 Stewart & Morin, 1926) This tissue, considered to be a chordal vestige, has been found to co-exist with malignant chor doma, but not in direct connection with it (Cappell, 1928)

The first case to be diagnosed *in vivo* was a cranial chordoma described by Klebs (1864) as a chordal chondroma.

Up to 1968 a total of about 550 cases have been reported (Wang & James, 1968) In Denmark Fabricius-Møller (1919) described the first nasopharyngeal case, and later 30 cases have been reported in Scandinavian literature including 3 nasopharyngeal ones. Godtfre

(1943) has described a case in which the cause of death was invasion of the carotid artery and Simonsen (1963) has reported a case in which death was due to subdural haemorrhage from a clivoid chordoma. The largest Scandinavian series, comprising 16 cases—3 lumbar and 13 sacrococcygeal—is Rosenquist & Saltzman s (1959)

Embryology

The notochord arises around the 3rd week of foetal life, developing first as the prochordal plate from Hensen s node which is situated in the centre of the embryonic disc. Hence it grows into the cranial direction by simple cellular proliferation up to Rathke s pouch at the spheno-occipital synchondrosis (Fig. 2) There is some disagreement as to whether it is to be considered endoderm or ectoderm. Therefore, some authors (Harvey & Dawson, 1941) have maintained that it is a tissue *sui generis* developing from its own blast, independently of the mesoderm by which it gradually becomes surrounded and which later makes up the vertebral body

According to Huber (1912) and Horwitz (1941), who studied foetuses with a view to the course of the notochord, it is quite close to the pharyngeal wall and in some cases embedded therein. Around the 8th week there eat developmental upheaval around the *of the notochord, and it has been imag*ed (Wright, 1967) that after this process some ectopic tissue might be left, giving rise to tumour formation.

Gradually as the vertebrae are completed, the notochord gets constricted, and remnants of it may be found in the nucleus pulposus, while otherwise it disappears. It is believed that the chordomas develop in intra-corporeal or extravertebral vestiges, while tumours have not been observed to develop from the nucleus pulposus (Harvey & Dawson, 1941)

Pathology

The tumour is firm, greyish-white, lobulated, encapsulated, and gelatinous on the cut surface When the capsule is removed there are 1–3 mm large vesicles, the so-called chordoma eyes" The tumour varies in size according to its site, the vertebral and cranial ones not exceeding a few cm, while in the sacral spine very large chordomas have been seen. The largest chordoma which has been removed weighed about 7 kg, and 19 months later the patient returned with a recurrence which weighed 11 kg (Mabrey 1935)

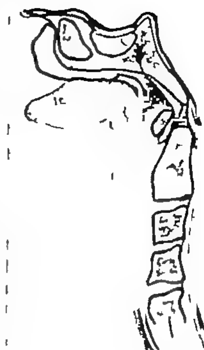


Fig 2 Course of the notochord.

The histological picture of this tumour is characterized by its variegated appearance, as the same tumour may include extremely cellular areas and areas which consist predominantly of extracellular accumulations of mucin. Between these extremes there are all kinds of transitional varieties. The tumour is divided into microlobules by fibrous connective-tissue strands which are vascular while the tumour tissue itself is avascular The cells are of extremely varied size, but of approximately the same shape. The smallest cells, peripherally in the lobules, are moderately eosinophilic and



Fig. 3. Periphery of tumour. Lobular structure. Fairly sharp demarcation against the surrounding muscles (inferiorly). 105

ly slightly vacuolated (Fig. 3). The nuclei vary little in size and chromatin density. As a rule they are centrally situated, but may be pressed peripherally by vacuoles so that the cells acquire the appearance of signet ring cells (Dahlin & MacCarty 1952). Moreover there may be nuclear vacuoles. Centrally in the lobes the cells fuse to a syncytium or to necrotic areas (Fig. 4). Extracellularly there will be accumulation of mucinous masses.

By electron microscopy Friedman *et al.* (1962) have found that there are two, possibly three kinds of cell, viz. stellate, physaliphorous, and transitional varieties, indicating that the physaliphorous ones develop from the stellate forms by vacuolation.

Histochemically several staining methods have been used as an aid in diagnosis. Primarily a number of methods for demonstrating mucus and also Best's carmine staining for demonstrating glycogen which is said to be accumulated especially in an intracellular situation. In the present case it was not possible to use the latter as the tissue had been fixed in aqueous formalin. All tests using mucicarmine were negative for intracellular mucus, while faint staining of extracellular mucus was obtained. Periodic acid-Schiff (PAS) gave the strongest positive reaction intracellularly while Alcian blue was positive both for extra- and intracellular mucus, but not equally strong in all cells, in particular not the largest. Like

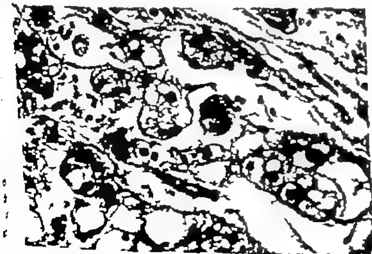


Fig. 4. Centre of tumour. Monstrous, coarsely vacuolated tumour cells with ample intercellular substance. 260.

Iurato & Leonardelli (1956) we found metachromasia extracellularly upon staining with toluidine blue, indicating that the ground substance is made up of acid mucopolysaccharides.

In spite of the characteristic histological picture, there have been, in the course of time, great difficulties in making the diagnosis at the first microscopic examination, probably because the tumour has not been borne in mind. The most common erroneous diagnoses have been myxosarcoma, chondroma, chondrosarcoma, mucinous adenocarcinoma, and myxochondro-osteoid osteoma or chondro-osteofibro-myxosarcoma (Mabrey 1935).

Metastases have been found in 10–15% of the cases, mainly from the sacral chordomas, possibly because on the whole they have existed for a long time before they are detected and treated. The metastases have been seen in the liver lungs, heart, bones, serous membranes, lymph nodes, and in the subcutaneous tissue (Higinbotham *et al.*, 1967 Wright, 1967)

Site sex and age

Coenen, in 1925 suggested a classification of this tumour which is still in use:

(1) cranial—sphenoidal
clivoid
nasopharyngeal
(2) vertebral—
(3) sacrococcygeal—(*a*) antesacral
(*b*) sacral
(*c*) retrosacral.

In comprehensive analyses (Dahlin & MacCarty 1952 Higinbotham *et al* 1967 Mabrey 1935) about 55% of the chordomas have been found to be sacrococcygeal, 35% cranial, and the remaining 10% vertebral, mainly cervical. Twice as many males as females are affected. Most of the cranial and vertebral chordomas occur in the age range 30–45 years, while the sacral ones are most common from 50 to about 65 years of age (Dahlin & MacCarty 1952) However a case has been observed in a 7 month foetus, and chordomas have been described in 80-year-old persons (Mabrey 1935)

Clinical features

No symptoms or signs are pathognomonic of chordoma. Of the chordomas which affect the upper part of the spine and the nasopharyngeal space there are the primarily vertebral ones and those which issue from the spheno-occipital synchondrosis whence they grow down into the rhinopharynx. The former usually manifest themselves in pain, partly suggesting root pain, pareses, dysphagia, hoarseness, and possibly dyspnoea. The latter give rise to nasal obstruction, rhinolalia clausa, and secondarily purulent nasal discharge and possibly epistaxis (Jepsen, 1951 Wright, 1967) In addition, there will be the primarily intracranial symptoms which usually are unilateral at first, but gradually become bilateral, primarily ocular symptoms because of involvement of the abducent nerve, the other ocular-muscle nerves, and optic nerve Thereafter deafness of the perceptive type, signs of pituitary involvement, headache, and bulbar palsy (Brændstrup, 1943)

The prognosis of the cervical chordomas is poor the average survival time being 2–4 years. The course of the sacral ones is somewhat longer as already mentioned. Dahlin & MacCarty (1952) have published the case of a patient who lived for 23 years after the diagnosis had been made

The cause of death has seldom been reported, but in most cases there has been a question of local recurrence, increase in intracranial pressure, cachexia, aplastic anaemia due to radiotherapy and in addition the three cases of vascular invasion.

Diagnosis

The investigations which may be applied in this type of tumour are the usual radiological examinations, but none is pathognomonic except for Sosman's sign, which is a small, v-shaped notch in the clivus Blumenbachii (Sø-

rensen, 1937) but this notch is evident only in the earliest cases and disappears gradually as the tumour destroys ever more bony tissue. In some cases, however it is possible to deter mine the extent of the tumour by tomography but in the great majority of cases the X ray examinations are entirely negative as in the present case where bone destruction was not visible until 4–5 months before death. Among the differential diagnostic possibilities there is tuberculosis of bone as well as the large number of primary or secondary tumours which may be situated in the skull or in and around the spine.

The only possibility of making the correct diagnosis is, as already mentioned, *histological* examination.

Treatment

The treatment is either surgical, radiological, or a combination of both. It is very difficult to remove the tumour radically unless, as suggested by some authors (Higinbotham *et al* 1967) the operation is hemicorporectomy with all its implications or removal of at least 2 vertebrae above the level where destruction may have been seen. Of course, this is applicable only for sacral chordomas, while in the case of vertebral or cranial chordomas repeated curettage of the tumour may be tried or possibly laminectomy without any hope of removing the tumour in healthy tissue. However when the operation is combined with heavy irradiation good palliation may be obtained.

Among patients treated exclusively by radiotherapy there have been cases in which doses up to a total of 17,500 R, administered as high-voltage irradiation, have resulted in a temporary but complete regression of all symptoms, without burn injuries (Boyle & Frank, 1966) Furthermore by the use of stereotactic apparatus, radioactive Yttrium seeds have been placed in the tumour Owing to its emission of beta-rays only this element can deliver a necrotizing dose locally without damaging the adjacent tissue (Zoltán & Fényes, 1960)

According to most recent investigations, the optimal tumour dose is about 6 000 R, and the previous opinion that this tumour was fairly radioresistant was probably due to the inadequate therapeutic possibilities.

ZUSAMMENFASSUNG

Es wird über einen Fall von Chordoma cervicalis bei einer 54-jährigen Frau berichtet. Er ist gekennzeichnet durch die Lokalisation des Tumors und den bis zur terminalen Phase subjektiv symptomlosen V rlauf sowie durch die Todesursache, die eine massive subdurale und subarachnoidale Blutung im Verlauf der ganzen Medulla spinalis war durch eine Arrosion der A. vertebralis verursacht.

Bei der Durcharbeitung der Literatur wurden keine Symptome gefunden, die patognomisch, geschweige denn charakteristisch für Chordome — weder kranîale, zervikale noch sakrale — sind und der Verlauf ist je nach der Lokalisation verschieden, indem die Überlebensdauer für die kranialen und zervikalen 2–4 Jahre ist, während sie fur die sakralen im Durchschnitt 5–7 Jahre ist.

Die charakteristische Histologie, die den alleinigen Weg zum Stellen einer exakten Diagnose darstellt, wird besprochen und der Verfasser hat ausserdem versucht, den Tumor histochemisch mit verschiedenen Schleimfärbungen zu kennzeichnen und gefunden, dass die Grundsubstanz aus sauren Mucopolysacchariden aufgebaut ist.

Addendum

Just as this paper was ready for print, case of nasopharyngeal chordoma was admitted.

This patient was 10-year-old boy with a history f nasal stenosis for about 1 year Most recently rhinolalia clausa, snoring, and mild, intermittent headache. Examination revealed on the right firm tumour the size of tangerine, not adhering to the mucosa, extending from the orifice of the Eustachian tube to about 2 cm beneath the soft palate. X-rays showed no definite signs of destructive changes in the bones. Operation disclosed an irregular intumescence, fairly adherent to the fascia. Inferiorly it could be detached entirely while superiorly its base formed small excoriation of the bone, so that chordoma eye" seemed to remain. After this cavity had been cleaned with sharp spoon, it was evident that spongy bone was not exposed. The postoperative course was largely uneventful. Histological examination of the removed tumo showed chordoma.

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FOOD AUDIOMETRY

Preliminary Report

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From the State Hearing Rehabilitation Center Copenhagen Denmark

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A procedure of combined visual and tangible reinforcements in behavioral audiometry by means of a food dispensing machine connected to the audiometer has proved to be successful in the examination of hearing in small children and in mentally retarded children.

In all kinds of animal experiments with mice, cats, dolphins, horses, etc., it is possible to condition the animal to perform all kinds of complicated manoeuvres, when they are rewarded by a little something to eat. This early primitive reaction we have made use of in conditioned pure tone audiometry with small children and mentally retarded children—and so far with great success.

APPARATUS

The equipment is composed of either a clinical audiometer or a portable audiometer connected with headphones or two loud-speakers placed on each side of the child. The tone stimuli can be activated from a pedal at the examiner's foot, so that movements of the hands do not influence the child.

The food-dispensing-machine consists of a dish of stainless steel. In the side of this dish is a narrow slit, which opens when the little wooden bear is pushed backwards. Inside the box is a magazine (like the one in an automatic gun). The magazine is filled with around 50 little chocolate tablets, which melt rapidly in the mouth and are swallowed quickly

By means of a switch, it is possible to make the eyes of the little bear glow at the same time as the pure tone is given over the audiometer This procedure is only used in the beginning of the test in order to attract the child's attention to the wooden bear Later on the illuminated eyes are switched off so that the child only reacts to the pure tones

If the child happens to push the bear backwards without a tone being given, the slit in the dish does not open, and the chocolate tablet cannot be ejected



Fig 1



Fig 2.

PROCEDURE OF EXAMINATION

If the child refuses to have the head-phones on, the child is placed between two loud-speakers and the food-dispensing-machine is placed on a table in front of the child. The examiner releases a pure tone of 1 000 Hz of a reasonable intensity by pressing his foot against the pedal. When the child hears the tone, the light glows in the bear's eyes The little patient gives the bear a push on its nose, and to the surprise of the child a chocolate tablet jumps out into the dish The tablet is grasped and soon eaten the child is then ready for the next tone impulse.

Due to the interest of children in this examination, we have obtained reproducible results from children down to the age of 1 year and even severely retarded children have shown such interest that it has been possible to find their pure tone thresholds, when this had not been possible with earlier procedures.

ZUSAMMENFASSUNG

Man hat einen Bonbon Automaten konstruiert, bei dem der Auslösungs-Mechanismus von einem Bären ausgeht, dessen Augen leuchten können. Von einem Audiometer gibt man einen Ton, worauf das Kind wenn es den Ton durch den Kopfhörer hören kann, dem Bären einen Druck auf die Nase gibt, wodurch wiederum ein Bonbon ausgelöst wird. Das Gerät hat sich bei Tonaudiometrie von kleinen Kindern und geistig verzögerten Kindern als ein verstärkter konditionierter Reflex herausgestellt.

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